CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES

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Submitted By

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I hereby declare that the thesis entitled **"CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES"** submitted towards partial fulfillment of the requirements for the award of **MD in Microbiology** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Veenu Gupta**, Professor & Head, Department of Microbiology, **Dr. Anil Kumar Kashyap**, Associate Professor, Department of Pulmonary Medicine, and **Dr. Jyoti Chaudhary**, Associate Professor, Department of Microbiology. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated

to

My Mother-in-law

Jagjit Kaur,

My Husband

Sanjamjit

&

My Son

Rehaanjit

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Dr. Bavleen Kaur

LIST OF ABBREVIATIONS

ABPA	Allergic bronchopulmonary aspergillosis
IPA	Invasive pulmonary aspergillosis
COPD	Chronic obstructive pulmonary disease
TB	Tuberculosis
CNPA	Chronic necrotizing pulmonary aspergillosis
CPA	Chronic pulmonary aspergillosis
IA	Invasive aspergillosis
IMF	International Monetary Fund
FDA	Food and Drug Administration
GM	galactomannan
BDG	β-D-glucan
BAL	Broncho alveolar lavage
PCR	Polymerase chain reaction
CT	Computed topography
IBPA	invasive bronchial-pulmonary aspergillosis
EORTC	European Organisation for the Research and Treatment of Cancer
MSG	Mycoses Study Group
PEG	Polyethylene glycol
TNF	Tumor necrosis factor
ET	Endotracheal
KOH	potassium hydroxide
SDA	Sabouraud dextrose agar
LCB	lactophenol cotton blue
ELFA	Enzyme Linked Fluorescence Assay
ECL	Electrochemiluminescence
EIA	enzyme immunoassay

ABSTRACT

Introduction - Aspergillus is one of the most ubiquitous airborne saprophytic fungi. Humans constantly inhale numerous conidia of this fungus. The conidia are normally eliminated in the immunocompetent host by innate immune mechanisms. With increase in the number of immunocompromised patients, there has been a dramatic increase in severe and fatal invasive aspergillosis.

Objectives-

1. To correlate mycological and serological profile of suspected pulmonary Aspergillosis cases.
2. To study the clinical spectrum and characterization of pulmonary Aspergillosis.

Materials and methods- The present study was a prospective study conducted over a period of one year in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana.

Samples like sputum, Bronchoalveolar lavage (BAL), endotracheal secretions, bronchial brushings, specimens obtained by needle aspiration or biopsy and blood (for serological analysis) from patients suspected of pulmonary aspergillosis were included in the study.

Results- This prospective study was conducted in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. Over a period of 1 year, a total of 89 cases with clinical suspicion of pulmonary aspergillosis admitted to various wards and ICUs of DMC&H were included in the study. The cases were categorized on the basis of clinical/ radiological/ serological and mycological profile. The mean age group affected was 58.7 yrs. Majority were males (60.6%) as compared to females (39.3%). Most common presentation was cough (89.9%) followed by chest pain (57.3%) and fever (56.2%). Out of 89 samples subjected to fungal culture, 55 (61.8%) showed fungal growth while 34 (38.2%) showed no growth. Most common chest X-ray finding was Homogenous Haziness (40.4%).Galactomannan assay positivity among suspected patients of pulmonary aspergillosis was 56 %. (1-3)-β-D glucan assay positivity among suspected patients of pulmonary aspergillosis was 83.3 %. The mortality in suspected patients of invasive aspergillosis was observed in 21.4% patients.

Conclusion –The study demonstrated that galactomannan assay and (1-3)-β-D glucan assay are useful diagnostic modality with a high sensitivity and specificity for the detection of pulmonary aspergillosis in high risk patients along with positive fungal culture. Thus, these tests are early markers for invasive aspergillosis.

Keywords- Fungal smear, Fungal culture, ABPA(Allergic Bronchopulmonary Aspergillosis), Invasive aspergillosis, Aspergilloma.

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INTRODUCTION

Fungal infections have recently emerged as a world-wide health care problem, because of extensive use of broad spectrum antibiotics, immunosuppressive agents and increasing population of terminally ill and debilitated patients [1]. *Aspergillus* infection is the commonest invasive fungal infection which involves respiratory tract. Man acquires this infection through inhalation of fungal spores, though infection has also been documented following exposure to water aerosols contaminated with *Aspergillus* spores and *Aspergillus fumigatus,* in particular, is the most important air borne saprophytic fungus [2]. Inhalation is the commonest mode of infection, lung is the most common site of involvement and pneumonia is the most important nosocomial infection caused by *Aspergillus* [3].

Depending upon the host immunity, lung structure and degree of inoculums, the spectrum of pulmonary disease ranges from a non-invasive disease (colonization or **aspergilloma**), or an allergic bronchopulmonary aspergillosis (**ABPA**), to a semi-invasive (**chronic necrotizing pneumonia**) or an invasive infection **(invasive pulmonary aspergillosis)**[4].

Pulmonary aspergillosis is seen mostly in immunosuppressed individuals. The central cause is the impairment of phagocytic system and hence, patients with haematological disorders are particularly prone to develop it. Diabetes mellitus, alcohol abuse, renal and hepatic failure, etc. which have an effect on neutrophil proliferation, maturation, function and lifespan, are the important predisposing factors [5].

A high index of suspicion is required in patients without the classical risk factors as early presentation is usually silent and non-specific, pyrexia uncommon and timely treatment is crucial for survival. Invasive aspergillosis has also been diagnosed in normal hosts after massive exposure to fungal spores. Chronic pulmonary aspergillosis affects patients without obvious immune compromise, but with an underlying lung condition such as COPD or sarcoidosis, prior or concurrent TB or non-tuberculous mycobacterial disease [6].

Aspergilloma is the most common and best-recognised form of pulmonary involvement by *Aspergillus* species, and it usually develops in a pre-existing cavity in the lung. The aspergilloma (fungus ball) is composed of fungal hyphae, inflammatory cells, fibrin, mucus, and tissue debris[7]. Many cavitary lung diseases are complicated by aspergilloma, including tuberculosis, sarcoidosis, bronchiectasis, bronchial cysts and bullae, ankylosing spondylitis, neoplasm, and pulmonary infection [8,9]. The fungus ball may move within the cavity, but it does not usually invade the surrounding lung parenchyma or blood vessels [10, 11].

The lesion remains stable in the majority of cases, but it may decrease in size or resolve spontaneously without treatment in 10% of cases. Rarely, the aspergilloma may increase in size [12]. Risk factors for poor prognosis of aspergilloma include the severity of the underlying lung disease, increase in size or number of lesions as seen on chest radiographs, immunosuppression (including corticosteroid therapy and HIV infection), increasing *Aspergillus*-specific IgG titres, recurrent large volume haemoptysis and underlying sarcoidosis [13].

Aspergillomas can often be diagnosed incidentally in an asymptomatic or minimally symptomatic patient and there is a significant risk of evolving complications. For patients with normal respiratory function, and with aspergilloma involving only one lung, 30-day postoperative mortality is anywhere between 1 to 5 %. Antifungal therapy alone is, on the other hand, of limited benefit in treating either simple or complex aspergilloma[14,15].

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder that results from a hypersensitivity reaction to the fungi *Aspergillus* antigen and presents with pulmonary infiltrates and bronchiectasis. A hypersensitivity reaction is evoked by repeated inhalation of *Aspergillus* spores. These *Aspergillus* spores become trapped in the thick sputum of patients causing a cascade of inflammatory reactions that can result in ABPA [16]. The hypersensitivity reaction elicited is mainly type I otherwise known as an immunoglobulin

E (IgE) mediated reaction [17]. Both type III and type IV hypersensitivity reactions have also been seen but less commonly [17].

ABPA is commonly associated in patients presenting with chronic bronchial asthma and in patients with cystic fibrosis but it can also occur in non-asthmatics. It is characterized by repeated episodes of exacerbation with periods of remission, if untreated may progress to fibrotic lung disease[18]. The disease is often incorrectly diagnosed as tuberculosis, bronchiectasis and bacterial pneumonia[19]. Almost half of the ABPA patients are initially misdiagnosed as pulmonary tuberculosis[20].

ABPA is suspected in any patient with asthma with lung shadows on chest skiagram and peripheral blood eosinophilia. All the features are not necessary to diagnose ABPA. The important diagnostic criteria are the presence of recurrent pulmonary infiltrates, peripheral blood eosinophilia and positive skin test to *Aspergillus* antigen[18]. There are five stages of ABPA: (i) acute, (ii) remission, (iii) exacerbation, (iv) corticosteroid-dependent asthma, (v) fibrotic lung disease. Early diagnosis and proper treatment can alter the prognosis of disease and may prevent prevent end stage lung fibrosis.

CNPA (semi-invasive aspergillosis) is an indolent cavitary infectious process of the lung parenchyma secondary to local invasion by a fungus of *Aspergillus* sp.[21]. This disease is most commonly seen in patients with altered local defences from pre-existing pulmonary disease, as well as in patients with mild immunosuppression[21,22]. CNPA is a diagnosis that should be considered in patients with a clinical presentation of pulmonary tuberculosis or in participants experiencing pneumothorax, especially in the context of corticosteroid treatment[23].

Invasive pulmonary aspergillosis is an aggressive disease due to the invasion of the bronchial wall and the accompanying arterioles by the hyphae. This form primarily occurs in severely immuno-compromised patients, especially patients with neutropenia due to allogenic bone marrow transplantation or chemotherapy for acute leukemia [24], but also patients who received solid-organ

transplantation, especially lung transplantation [25]. Symptoms are non-specific and usually mimic bronchopneumonia, but also include pleuritic chest pain and haemoptysis [7]. There are two sub-categories of invasive aspergillosis, the airway-invasive form and the angio-invasive form.

The airway-invasive form accounts for 15 to 30% of invasive aspergillosis, and is defined by the presence of *Aspergillus* deep to the basal membrane of the bronchi [26]. The angio-invasive form is histologically characterized by the invasion and occlusion of small to medium-size pulmonary arteries by fungal hyphae [27]. Typical CT findings [26], 27] consist in larges nodules surrounded by ground glass attenuation, which is called the “halo sign”. Nodules are due to coagulation necrosis, whereas the halo of ground glass is due to surrounding alveolar haemorrhage [28]. There are increasing numbers of reports documenting invasive aspergillosis in immunocompetent patients with severe COPD, often with prolonged use of corticosteroid therapy [7].

Beyond this segmentation in four forms it should be noted that an overlap between the main categories of *Aspergillus*-related diseases has been reported. Some forms could co-exist, especially allergic broncho-pulmonary aspergillosis and aspergilloma [7], [29].

Changes of local or systemic host immunity and of underlying lung pathology can modify aspergillosis pulmonary manifestations with time. For example, an aspergilloma can develop within bronchiectasis in a patient with ABPA. An invasive form can complicate long-standing ABPA in case of systemic immune deficiency, including the use of high-dose of steroids [29].

The correlation between the immunologic status of the host which is an essential factor in the occurrence of *Aspergillus*-related lung diseases, and the radiological presentation are of major importance for accurate diagnosis of these diseases.

AIMS AND OBJECTIVES

- To correlate mycological and serological profile of suspected pulmonary Aspergillosis cases.
- To study the clinical spectrum and characterization of pulmonary Aspergillosis.

REVIEW OF LITERATURE

Aspergillosis is a disease caused by fungi of the genus *Aspergillus* mainly *A. fumigatus*, and less commonly *A. flavus*, *A. terreus*, and *A. niger*. The name *Aspergillus* is derived from its resemblance to the sporulating head of *Aspergillus* and the aspergillum/aspergill used to sprinkle holy water (from latin aspergere: to sprinkle.) [30]. John-Hughes Bennet (1812-1875) was first to describe the aspergillosis. In his seminal paper, which was published in 1842 he made the very first description of *Aspergillus* growing in lung tissue of humans. In 1926, Tom and Church first classified genus and they accepted 69 *Aspergillus* species in 11 groups[31]. Raper & Fennell in 1965 recognised 132 species and 18 varieties and they provided Latin descriptions for new species but they did not designate the formal type specimens and instead they used type strains[32].

Aspergillus species are common saprotrophic in soil and on decaying plant materials and their spores are ubiquitous in environment. The spores of this ubiquitous mold are dispersed by wind in the atmosphere and its inhalation is the primary route of access in almost all forms of aspergillosis. *Aspergillus* species are characterized by the production of hyaline, dichotomous branching, septate hyphae with parallel walls. A specialized hyphal segment known as foot cell, serve as the base of origin of the conidiophores. Conidiophores terminate in a swollen vesicle, from the surface of which are borne one (uniseriate) or two (biseriate) rows of phialides[33]. Pulmonary aspergillosis is the usual clinical entity, which includes: CPA (including single pulmonary aspergilloma), allergic bronchopulmonary aspergillosis, subacute invasive (chronic necrotizing pulmonary), and acute invasive aspergillosis. Immunodeficiency and/or underlying lung disease are the major factors which leads to aspergillosis.

The saprophytic colonization of pre-existing cavitary spaces in pulmonary parenchyma, its clinical presentation, as well as its complications are referred to as aspergilloma. Description of cavitary aspergillus disease dates back to John Hughes Bennett in 1842,

whose description of the soft tuberculous matter in multiple varied sized cavities in the lung closely resembles the aspergillus disease. Deve in 1938 first formally described an aspergilloma and named it as “mega mycetoma”[34]. Subsequently, Hinson, and colleagues defined aspergilloma in its currently understood version of a saprophytic infection of pre-existing lung cavities[35].

Even though aspergillomas are often diagnosed incidentally in an asymptomatic or minimally symptomatic patient, there is a significant risk of evolving complications. Rafferty and colleagues noted that 20% of non surgically treated mycetoma progresses to invasive aspergillosis. Similarly, Paneth et al. observed that after an episode of hemoptysis, 5-year survival for operated patients was 41% compared to 84% for conservatively treated patients. A significant proportion of patients with aspergilloma have a pre-existing lung disease. Therefore resection with lung-sparing surgery has been gaining ground over the years.

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder that results from a hypersensitivity reaction to the fungi *Aspergillus fumigatus*. Dr. K.F. Hinson first described allergic bronchopulmonary aspergillosis (ABPA) in 1952 [35]. Several years later in 1968 ABPA was first reported in the United States [19]. This caused an increased awareness of ABPA. Greenberger and Patterson studied 531 asthmatic patients from the United States between 1983 and 1986[19]. They found that 32 patients or 6% had immediate cutaneous reactivity to the *Aspergillus* antigens, which was the causative antigen in ABPA. ABPA should be ruled out in every case of uncontrolled asthma. Diagnostic criteria for ABPA include the presence of bronchial asthma, immediate skin test reactivity to *Aspergillus fumigatus*, elevated serum IgE levels, total and *Aspergillus fumigatus*-specific, pulmonary infiltrates (transient or fixed), central bronchiectasis, peripheral blood eosinophilia, and presence of serum precipitins against *Aspergillus* antigen[36].

Binder et al. defined the term chronic necrotizing pulmonary aspergillosis (CNPA) to describe the state between aspergilloma and invasive pulmonary aspergillosis. Binder et al. [37] proposed that CNPA is a disease pattern that is independent of the pulmonary aspergillosis that characteristically develops in patients with mild immunosuppression, including those with diabetes mellitus, malnutrition, long term use of low-dose corticosteroids, and collagen vascular diseases such as ankylosing spondylitis and rheumatoid arthritis. Although the clinical course is a chronic process that progresses slowly over several months to years, cavitations occur continuously due to hyphae invading into the tissues, and there is no vascular invasion or dissemination to other organs. This was followed by Gefter et al. [38], who defined semi-invasive aspergillosis as a lesion accompanied by destruction of the lung without fungus invasion of the tissues.

Ronen et al described the pathogenesis of IA and evaluated that the principal events take place in the alveolus, where inhaled conidia germinate into invading hyphae that penetrate the respiratory epithelium. Various Aspergillus traits, including the production of pigment, antioxidants, proteases, adhesins, siderophores, and mycotoxins, are the potential virulence factors[39].

Chakrabarti et al reviewed invasive aspergillosis (IA) in developing countries, included those countries, which are mentioned in the document of the International Monetary Fund (IMF), called the Emerging and Developing Economies List, 2009. Sub-optimum hospital care practice, hospital renovation work in the vicinity of immunocompromised patients, misuse of steroids and antibiotics and use of contaminated infusion sets have been reported their association with IA. Other risk factors include liver failure, COPD, diabetes, and tuberculosis are the newly recognized underlying diseases. In Asia, Africa and Middle East, sino-orbital or cerebral aspergillosis and *Aspergillus* endophthalmitis are emerging diseases and *Aspergillus flavus* is the predominant species isolated from these infections[40]. The conditions which are commonly associated with CNPA are: COPD,

previous pulmonary tuberculosis, atypical *Mycobacterium tuberculosis* infection, cystic fibrosis, pulmonary infarction, pulmonary fibrosis, previous surgery, and pneumoconiosis. Other conditions which may be associated with the development of CNPA include lung cancer, fibrocystic pulmonary sarcoidosis, silicosis, diabetes mellitus, malnutrition, alcoholism, connective tissue diseases, and prolonged corticosteroid therapy. Although a CT scan is helpful in the diagnosis of airway aspergillosis, diagnosis of this specific disease cannot be made on the basis of imaging features alone. Bronchoscopy, apart from providing specimens for microbiological examination, is a useful method for detecting infections with endobronchial manifestations[41]. It should be recommended as early as possible for diagnosis and to maintain airway patency, at a time when it is most feasible[41]. Early detection of intraluminal growth of *Aspergillus* and appropriate antifungal therapy can facilitate the management of these patients and can prevent the development of complications.

Invasive pulmonary aspergillosis (IPA) is the most severe among the diseases caused by *Aspergillus spp.* It affects mostly the immunocompromised individuals, and carries a high mortality rate ranging from 35% to 78% with high economic burden. IPA is characterized by the invasion of pulmonary arterioles and lung parenchyma by *Aspergillus* hyphae that leads to ischemic necrosis, intravascular thrombosis, hemorrhagic infarct from rapid hyphal growth [42]. The angioinvasion of distant organs via hematogenous dissemination frequently involves the kidneys, liver, spleen, and the central nervous system. Schwartzet al. evaluated the clinical presentation of Invasive Aspergillosis. The earliest sign of an invasive fungal infection is fever which is not responsive to broad-spectrum antibiotics. Symptoms include cough, dysnoea and chest pain which can be noted early in the course, whereas symptom like hemoptysis typically comes late. It may also involve the nasal cavity or sinuses resulting in nasal obstruction, epistaxis, facial pain, periorbital swelling and palate destruction. Other sites of infections include central nervous system resulting in focal neurological lesions. The recognition

of symptoms associated with invasive aspergillosis in patients at risk should be diagnosed as early as possible and immediate antifungal therapy should be started to improve the treatment outcome [43].

Chakrabarti et al reviewed invasive aspergillosis (IA) in developing countries, included those countries, which are mentioned in the document of the International Monetary Fund (IMF), called the Emerging and Developing Economies List, 2009. Sub-optimum hospital care practice, hospital renovation work in the vicinity of immunocompromised patients, misuse of steroids and antibiotics and use of contaminated infusion sets have been reported in the association with IA[40].

Recent advances of diagnosis of invasive aspergillosis include antigen detection tests; galactomannan and β-D-glucan (component of cellular wall). Both these tests are approved by the US Food and Drug Administration (FDA) for the diagnosis of IPA. They help in the early diagnosis and confirmation of IPA and are also helpful in the assessment of the evolution of infection during treatment with serial levels[44]. But the gold standard for diagnosis is via biopsy of lung tissue, in combination with positive culture of *Aspergillus* spp. from the biopsy specimen. However, patients with suspected IPA often have profound thrombocytopenia, or are clinically unstable to undergo biopsy procedures.

A Cochrane meta-analysis by Leeflang et al. conducted in immunocompromised patients demonstrated serum GM to have sensitivity varying between 78% to 63% and specificity of 85% to 93%, depending on the optical density index (ODI) used as cut off from 0.5 to 1.5 respectively [45]. A meta-analysis by Hou et al. included 1068 patients from 11 studies found the sensitivity and specificity of the diagnostic accuracy of serum BDG to be 75% (64–84%, 95% CI) and 87% (81–92%, 95% CI) respectively as a screening tool for invasive fungal disease [46]. Combining serum GM with serum beta-D-glucan (BDG) or having two positive BDG test consecutively will significantly increase the specificity. The increased specificity in two consecutive tests indicate value of using PCR as a diagnostic screening tool. Avni

et al. demonstrated higher summary sensitivity and specificity of BAL PCR of 90% (77–96%, 95% CI) and 96% (93–98%, 95% CI) respectively [47].

Radiographic imaging is frequently used to aid in diagnosis of IPA. HRCT is the preferred method at the early stages to identify lesions of IPA, with features including nodules, consolidative lesions, and wedge-shaped infarcts. Characteristic feature of IPA, especially in neutropenic patients, is the 'halo sign', which is a central nodule that's > 1 cm in diameter surrounded by a ground-glass opacity reflecting hemorrhage. Halo sign is seen only during the early stages of angioinvasion, and thus can be easily missed if CT scan is not done during this window. The 'air crescent sign' seen in ~50% of cases usually two weeks after disease progression, after the patient's immune system has recovered from the initial insult. It is a crescentic area of radiolucency within the nodular opacity [48]. Due to the potential for disease progression, it is recommended that treatment should be initiated early in cases of high suspicion. The therapeutic response can be monitored through the evaluation of the signs, symptoms and radiological findings at regular intervals. Surgical resection should be considered in cases of lesions that are in proximity with the great vessels or with the pericardium or with the chest wall.

The retrospective study was conducted in 91 patients for exploring the impact of the neutrophil count on the GM serum index at the time of IA diagnosis. Ninety-one patients of IA with haematological malignancies were studied. Three groups were identified: groups 1–3, with <100 polymorphonuclear neutrophils (PMN)/mm3 (n = 18), between 100 and 500 PMN/mm3 (n = 21), or >500 PMN/mm3 (n = 60), respectively. The mean GM index was significantly higher in group 1 than in the other groups ($p < 0.05$) [6].

A study was conducted recently on the extrapulmonary invasive aspergillosis. Two patients develop progressive intra-abdominal aspergillosis but both patient had not developed *Aspergillus* infection of the lungs. One patient had homozygous M1I *CARD9* (caspase recruitment domain family member 9) mutation, while the other had

homozygous Q295X *CARD9* mutation, both patients lacked CARD9 protein expression. CARD9 deficiency is the first known inherited or acquired condition that predisposes to extrapulmonary *Aspergillus* infection with sparing of the lungs [49].

Kurhade et al conducted a study on the prevalence and predisposing factors of *Aspergillus* infection and correlate microscopy, culture and serological findings. *Aspergillus* species were isolated in 20 (16.26%) cases and *Aspergillus* fumigates was the predominant species 16 (80%). Precipitins were detected in 29 (23.58%) cases. Serological tests have an edge over routine smear and culture methods for the diagnosis of pulmonary aspergillosis and fluconazole in the treatment of aspergillosis [50].

Munoz et al stated that the diagnosis of aspergillosis is based on the combination of compatible clinical findings with risk-factors and isolation of the microorganism, radiological findings, serological detection of antibodies or antigens, or histopathological evidence of invasion. Chest radiographic findings may initially be normal in up to 10% of cases. Computed tomography scanning is probably the most useful imaging technique as it may reveal lung lesions earlier. Non-invasive technique could be used as markers of infection of *Aspergillus* which are galactomannan (GM) and (1-3)-ß-D-glucan (BDG). GM has a high specificity of 85% and sensitivity varies between 30% and 100%. BDG has a sensitivity ranging from 50% to 87.5%. A specific Aspergillus PCR assay has also been used in the diagnosis of IA and has a sensitivity and specificity of 100% and 89%, respectively [51].

A retrospective cohort study was conducted to evaluate the value of radiological examinations in the diagnosis and prognosis of invasive bronchial-pulmonary aspergillosis (IBPA). Out of 61 patients, 21 were in IBPA group and 40 in non-IBPA group. The chest CT image analysis showed that IBPA group had highest percent of patchiness (76.2%), followed by multiple nodules and angio-invasive patterns including halo sign, wedge consolidation and air-crescent sign. Deteriorated chest X ray combined with specific laboratory examinations could indicate a diagnosis of IBPA [52]. Maertens et al

conducted a study on 100 consecutive patients to evaluate the relationship between antigenemia and other diagnostic triggers. Galactomannan detection had a sensitivity of 94.4% and specificity of 98.8%. This statistical profile was better than that of other triggers, including unexplained fever, isolation of *Aspergillus* species, and abnormalities seen on radiological examination. Antigenemia preceded diagnosis on the basis of radiologic examination or *Aspergillus* isolation by 8 and 9 days in 80% and 88.8% of patients, respectively. Prospective screening for GM assay allows earlier diagnosis of aspergillosis than the conventional diagnostic criteria [53].

IA have greatly benefited from the European Organisation for the Research and Treatment of Cancer (EORTC) and US Mycoses Study Group (MSG) criteria for defining invasive fungal infections. The revised definitions retain the original classifications of “proven,” “probable,” and “possible” invasive fungal disease, but the definition of “probable” has been expanded, whereas the scope of the category “possible” has been diminished [27].

Several factors affect the accuracy of the test. Aubry et al stated that antibiotics may cause false-positive results. The greatest problem has seen in patients on piperacillin-tazobactam, amoxicillin or amoxacillin-clavulate [54]. In another study, they described that positive result may occur in patients infected with fungi containing a cross reactive galactomannan. The monoclonal antibody used reacts with β-1-5-linked galactofuranose in the galactomannan of *Penicillium, Paecilomyces, Alternaria, Trychophyton, Botrytis, Wallemia, Cladosporium* [55]. Similar findings were observed in another study that the treatment with antifungal drugs with activity against *Aspergillus* reduces the sensitivity of GM assay [56]. Although antifungal therapy reduced sensitivity, it did not eliminate the utility of the assay for diagnosis of aspergillosis [57].

W. Steinbach and Stevens evaluated that previously there were only 2 antifungals with activity against Aspergillus but in recent years there has been introduction of newer agents and reformulations of older antifungals which are cyclodextrin-itraconazole, liposomal

nystatin, and PEG-amphotericin B. Exploration has also begun with immunotherapy, cytokines and granulocyte transfusions alone or in combination with antifungal therapy [58].

In a study conducted by Shirkhani et al, they analyzed the consideration of pre-exposure prophylaxis to prevent invasive aspergillosis infection. They study a very low molecular weight amphotericin B-polymethacrylic acid nanoparticle. Protection from disease was associated with >99% killing of the *Aspergillus* and a 90% reduction in lung TNF-α; the primary driver of tissue destructive immuno-pathology [59].

Mayr et al evaluated the emergence of resistance to azoles which was found mainly in *Aspergillus fumigatus*. This may be due to long term azole therapy in patients with chronic aspergillosis. The primary mechanism of resistance is due to alterations in the cyp51A target gene, with a variety of mutations found in clinical isolates and one genotype identified was lH98. *Aspergillus* species and resistance may be under-diagnosed as susceptibility testing is less frequently performed due to technical reasons [60].

There are many unanswered and unresolved epidemiological, laboratory, and clinical questions that need to be addressed and understood in the diagnosis, treatment, and prevention of aspergillosis. Better diagnostic tests are needed to facilitate more accurate identification of patients with invasive aspergillosis and to permit earlier initiation of therapy. The availability of more-active and better-tolerated antifungal agents has significantly improved therapy of patients at risk for serious *Aspergillus* infection. However, critical gaps in knowledge remain regarding management of these infections, including the use of combination therapy, tools for early detection of these infections, evaluation of response, therapy for patients with breakthrough or refractory infection, and the patient population for whom prophylaxis would be most beneficial.

MATERIAL AND METHODS

The present study was a prospective study conducted over a period of one year in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana.

Samples like sputum, Bronchoalveolar lavage (BAL), endotracheal secretions, bronchial brushings, specimens obtained by needle aspiration or biopsy and blood (for serological analysis) from patients suspected of pulmonary aspergillosis were included in our study.

METHOD OF COLLECTION OF DATA

The data collected was entered in a semi-structured proforma which included patient's details: Name, age, gender, CR. No., Lab.ID no., date of admission, provisional clinical diagnosis, risk factors, treatment history, haemogram, radiological evaluation, histopathological evaluation, microbiological evaluation (Galactomannan assay, (1-3)-β-D glucan assay, fungal smear/culture), clinical outcome.

SOURCE OF DATA

Samples received from suspected patients of Invasive Aspergillosis admitted in various wards and ICUs of Dayanand Medical College and Hospital, Ludhiana were included.

Sample Collection

- **Blood:** Five ml blood was collected by venipuncture in sterile plain vials taking all aseptic precautions.
- **Sputum:** Sputum was collected in a wide mouthed, sterile, leak-proof, screw capped container under all aspectic precautions [61].
- **Bronchoalveolar Lavage (BAL):** Sample was collected using flexible fiberoptic bronchoscope. About 30-50 ml of physiological saline was infused through fiberoptic bronchoscope into peripheral bronchiolar ramification. The saline was then aspirated and collected in a sterile, robust and leak proof container[33].

- **Endotracheal (ET) secretions :**. ET secretion was collected using a 22-inch Ramson's 12 F suction catheter with a mucus extractor which was gently introduced through the endotracheal tube for a distance of approximately 25–26 cm. Gentle aspiration was then performed without instilling saline and the catheter was withdrawn from the endotracheal tube. About 2 ml of sterile 0.9% normal saline was injected into it with a syringe to flush the exudates into a sterile container for collection[51].

Sample Processing:

1. Microscopic examination and culture of fungus

I. Direct microscopic examination [31,51]

Potassium hydroxide mount: -

Composition of KOH:-

Potassium hydroxide	10 gm
Glycerol	10 ml
Distilled water	80 ml

These ingredients were mixed properly and solution was stored at room temperature.

Slide KOH preparation:

A drop of potassium hydroxide solution was placed on a clean microscopic slide. The sample was transferred to the drop of KOH and mixed gently with inoculating loop. A cover slip was placed over it. Clearing was hastened by gently heating the preparation over the flame of a Bunsen burner, taking care to prevent drying. It was then examined microscopically under low (10×) and high (40×) power objectives.

Tube KOH preparation:

Tube KOH method was used for biopsy specimens which take longer time for dissolution. The tissue was put in 10-20% KOH and was kept in an incubator at 37°C for dissolution. It was then examined microscopically under low (10×) and high (40×) power objectives.

Presumptive identification was made based on direct microscopic examination of material from clinical sample. Hyaline septate fungal

hyphae with acute angle branching were suggestive of *Aspergillus* species.

i. Fungal culture:[31]

Culture media used were:

a) **Sabouraud dextrose agar (SDA) with antibiotics and without cycloheximide**

Composition and preparation:

Peptone	10 gm
Dextrose	40 gm
Agar	20 gm
Distilled water	1000 ml
Chloramphenicol	50 mg
Gentamicin	20 mg

All ingredients were dissolved by boiling; antibiotics were added into SDA while boiling but before autoclaving. Chloramphenicol/ gentamicin were dissolved in 10 ml of 95% alcohol and added to boiling medium. The medium was dispensed in tubes and autoclaved at 121ºC for 15 minutes and final pH was adjusted to 5.6. The medium was allowed to cool in slanting position and stored in the refrigerator.

b) Sabouraud dextrose agar (SDA) with antibiotics and cycloheximide

All ingredients of SDA with antibiotics were mixed properly and following antibiotic was added while boiling but before autoclaving:

Cycloheximide 500mg/1000ml

Cycloheximide was dissolved in 10ml acetone, added to boiling medium and mixed properly. The medium was removed from heating and mixed well. It was dispensed in tubes and autoclaved at 121ºC for 15 minutes and final pH was adjusted to 5.6. The medium was dispensed in tubes and allowed to cool in slanting position and stored in the refrigerator.

Processing of sample for fungal culture:-

The samples were inoculated on two tubes of Sabouraud dextrose agar (SDA) with antibiotics (chloramphenicol 50mg/1L and gentamicin 20mg/1L) and cycloheximide (500mg/1L) and two tubes of SDA with antibiotics but without cycloheximide. One set of tubes was incubated at 25ºC. Similarly, other set were incubated at 37ºC. The inoculated media were kept for a minimum period of three weeks. Tubes were examined daily during 1st week and on alternate days in 2nd and 3rd week. The growth obtained was identified on the basis of colony morphology, pigment production and microscopic examination of lactophenol cotton blue (LCB) preparation.

- **Lactophenol cotton blue mount preparation:**[31]

  Composition and preparation

Phenol crystals	20 ml
Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml
Cotton blue	0.05 gm

  All reagents were mixed properly and cotton blue stain was dissolved in distilled water before mixing with remaining reagents.

- **Tease mount preparation**[33]

Growth on culture tubes was teased out on a glass slide in a drop of LCB stain using two teasing needles. Coverslip was placed over it and examined under microscope to study morphological features of fungal isolates.

- **Transparency Tape method of preparing LCB mount**[33]

A drop of LCB was placed on a clean microscope slide. A piece of clear vinyl adhesive tape 6-7 cm long was held with forceps and introduced into the culture tube. The centre of the adhesive side of the tape was allowed to touch the colony lightly under its own weight. The tape was transferred to the slide, placing the area with adhered

mycelium on to the lactophenol cotton blue. Growth of *Aspergillus* species was taken as significant and processed for identification

Microscopic examination revealed spores and other relevant structures.

Fungal growth	Colonies	Reverse	Conidiophores	Phialides
A. flavus	Velvety, yellow to green or brown	Golden to red brown	Rough, pitted and spiny	Uniseriate & double cover entire vesicle
A.fumigatus	Velvety or powdery, turning to smoky green	White to tan	Smooth	Uniseriate/ biseriate, cover upper half vesicle
A.niger	Woolly, white to yellow, turning dark brown to black	White to yellow	Smooth	Biseriate, covering entire vesicle
A.terreus	Velvety, cinnamon brown	White to brown	Short, smooth	In two rows, compactly columnar
A.glaucus	Green with yellow areas	Yellowish to maroon	Smooth	Single, radiate to loosely columnar

- The patients were categorized into “proven,” “probable,” and “possible,” cases based on risk factors, microbiological and clinical criteria, as per Invasive Fungal Infections Co-operative

Group (IFICG) of the European Organization for Research and Treatment of Cancer and Mycoses study group case definitions (EORTC/MSG) [62].

2. Aspergillus skin test (Intradermal test)

It was performed using *Aspergillus* antigen kit commercially available from Alcure Pharmaceuticals (India). The test was performed as per the kit manual. The following antigens and controls were used,

Negative control: Phosphate buffer saline (PBS)

Positive control: Histamine acid phosphate in PBS (15 µg/ml)

Antigens: Antigens of *A. flavus, A. fumigatus, A. niger, A. tamarii* and *A. versicolor* were used (1:500 w/v concentrations)

Dose: 0.01 to 0.02 ml with tuberculin syringe

Procedure 0.01 to 0.02 ml of test antigens, buffered saline (negative control) and histamine (positive control) solution were injected intradermally on ventral aspect of the forearm raising bleb of 2 to 3 mm. Separate syringes were used for each antigen, Minimum distance kept between two injection sites was 5 cm to avoid overlapping of reactions.

Interpretation:

Tests were interpreted after 1 hour and then after 6 hrs. Test was read only when the negative control showed no wheal and erythema and the positive control was ≥6 mm.

Negative: When test appeared similar to negative control, it was labelled as negative.

Positive: Any test showing wheal more than positive control and erythema about 15 to 20 mm was taken as positive.

Type I: When wheal and erythema developed within a minute and reached maximum size after 10-20 minutes and resolved within 1-2 hrs, it was considered as type I reaction.

Type III: Any amount of subcutaneous oedema at the end of 6 hrs was considered as type III reaction.

3. Total IgE levels:

Serum sample was collected aseptically in a plain vial. The test was performed by Electrochemiluminescence (ECL) as per the kit protocol.

Interpretation:

>1000IU/ml were considered as significant levels for ABPA

4. Galactomannan assay:

Principle: It is a one-stage immunoenzymatic sandwich microplate assay which detects galactomannan in human serum. The assay uses rat EBA-2 monoclonal antibodies, which are directed against *Aspergillus* galactomannan.

Semi-quantitative determination of *Aspergillus* GM antigen in serum was done using Platelia Aspergillus EIA (enzyme immunoassay) kit (*Bio-Rad,* France) as per manufacturer's instructions.

Interpretation:

- Sera with an index < 0.50 were considered as negative for galactomannan antigen.
- Sera with an index ≥ 0.50 were considered as positive for galactomannan antigen.

Variables such as Mold active antifungal drugs (amphotericin B, voriconazole, itraconazole), piperacillin-tazobactam, amoxacillin-clavulanic acid, steroids, neutropenia were studied.

5. (1-3)-β-D Glucan Assay: The Fungitell kinetic assay is based upon the determination of the rate of optical density increase produced by a sample. The Fungitell test results are expressed in picogram/ml.

Interpretation:

NEGATIVE RESULT: <60 pg/mL

INDETERMINATE RESULT: 60 to 79 pg/mL

POSITIVE RESULT: ≥ 80 pg/mL

6. Chest X-Ray and High Resolution Computerized Tomography (HRCT):

Pulmonary aspergillosis can be classified as Invasive aspergillosis, ABPA and Aspergilloma. Clinical and mycological findings will be correlated in our study.

Invasive aspergillosis cases will be classified according to revised definitions given by EORTC/MSG Consensus group into proven, probable and possible invasive aspergillosis based on host, clinical and mycological factors.

Host criteria

– Diabetes

– Post stem cell transplantation

– Prolonged corticosteroid use

– Received immunosuppressants

– Inherited immunodeficiency

Clinical criteria Presence of 1 of the following three signs of radiology

– Dense, well-circumscribed lesion with or without a halo sign

– Air-crescent sign

– Cavity

Mycological criteria

- Positive indirect tests by galactomannan antigen detection
- Positive direct test by smear/culture

Diagnosis	Criteria+
Proven IA	Histopathological evidence of tissue invasion by filamentous fungi, Isolation of *Aspergillus* species from a normally sterile but clinically infected body site
Probable IA	One microbiological criteria, and One host criteria, and Clinical criteria
Possible IA	Host criteria, and Presence of microbiological criteria, or Clinical criteria

OBSERVATIONS AND RESULTS

This prospective study was conducted in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. Over a period of 1 year, cases with clinical suspicion of pulmonary aspergillosis admitted to various wards and ICUs of DMC&H were included in the study. Total of 89 samples were received from patients with suspected pulmonary aspergillosis,89 samples were received for fungal smear and fungal culture, 50 samples were received for galactomannan and 30 for (1-3)-β-D glucan assay. Smear positivity was 55%, culture positivity was 61.8%, galactomannan positivity was 56% and fungitell positivity was 83.3% in enrolled cases. The cases were categorized on the basis of clinical/ radiological/ serological and mycological profile. 4 patients were labelled as cases of ABPA, 75 were cases of Invasive Aspergillosis, 4 were fungal colonizers and 6 were of no fungal infection. Cases of Invasive aspergillosis were further characterized into Possible (84.3%), Probable (77.5%) and Proven (0%).

Table 1: Age wise distribution of suspected pulmonary aspergillosis cases (n=89)

Age Group(in years)	Total number of patients (%)
20-30	3 (3.4)
31-40	7 (7.9)
41-50	4 (4.5)
51-60	34 (38.2)
61-70	23 (25.8)
71-80	15 (16.9)
>80	3 (3.4)

In the present study, suspected cases of pulmonary aspergillosis were most commonly seen in the age group of 51 to 60 years.

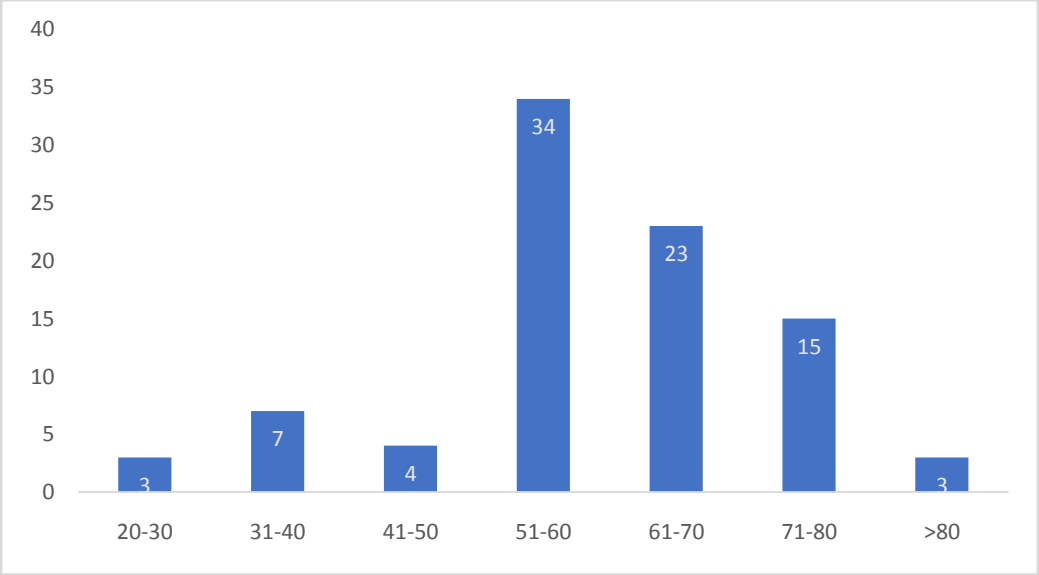

Figure 1: Age-wise distribution of suspected pulmonary aspergillosis cases (n=89)

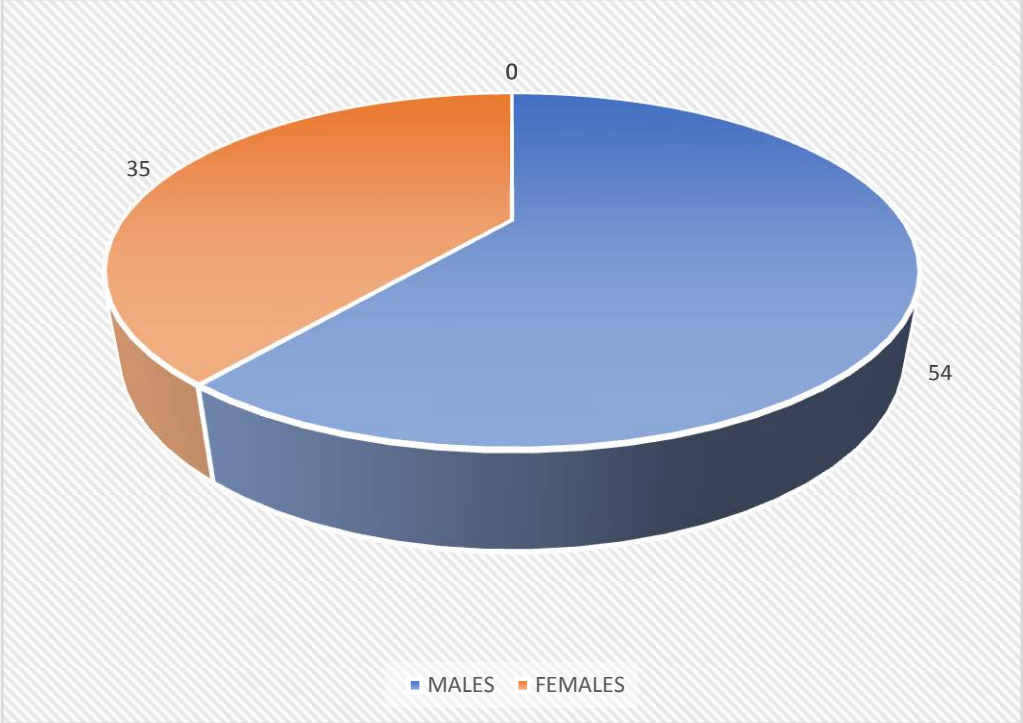

Figure 2: Sex-wise distribution of suspected pulmonary aspergillosis cases (n=89)

Out of a total 89 cases of suspected pulmonary aspergillosis, 54 (60.6%) patients were males and 35 (39.3%) were females.

Table 2: Distribution of risk factors in suspected patients of pulmonary aspergillosis (n=89)

Risk factors	Total (%)
Diabetes	45(50.6)
Chronic steroid use	36 (40.4)
Malignancy	3 (3.4)
HIV infection	3 (3.4)
Solid organ transplant recipients	2 (2.2)

Most common risk factor among suspected patients of pulmonary aspergillosis was diabetes (50.6%) followed by chronic steroid use (40.4%).

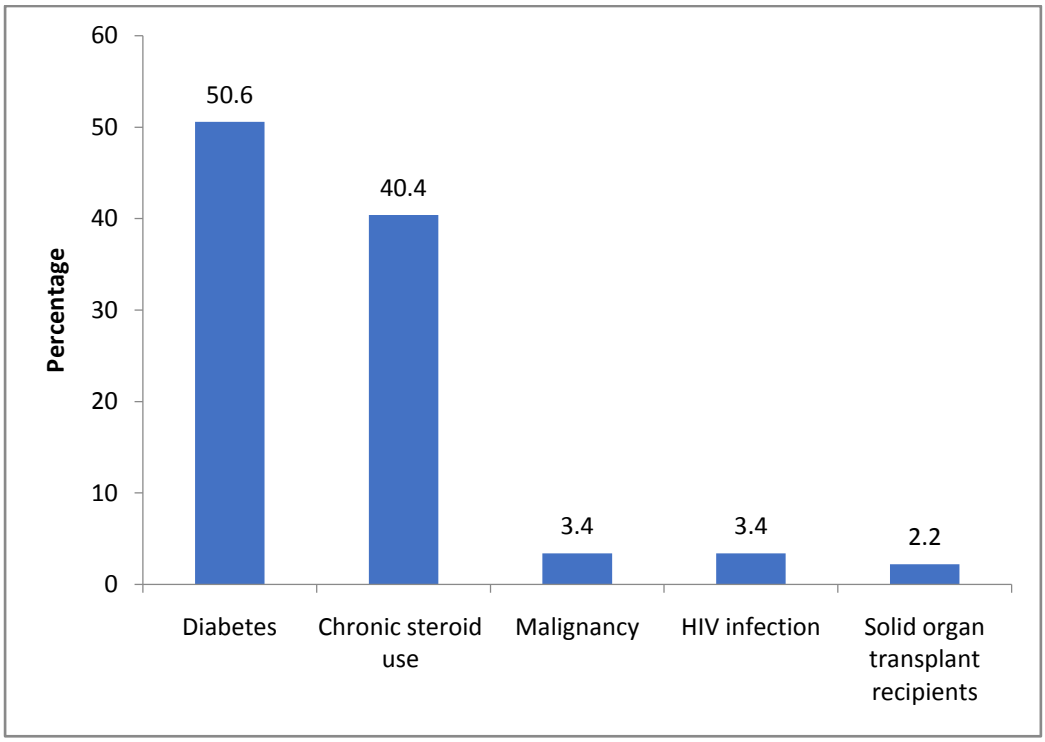

Figure 3: Distribution of risk factors in suspected patients of pulmonary aspergillosis (n=89)

Table 3: Distribution of suspected patients of pulmonary aspergillosis on the basis of clinical symptoms (n=89)

Symptoms	Total (%)
Cough	80 (89.9)
Chest pain	51 (57.3)
Fever	50 (56.2)
Dyspnoea	45 (50.6)

Most common presentation was cough (89.9%) followed by chest pain (57.3%) and fever (56.2%).

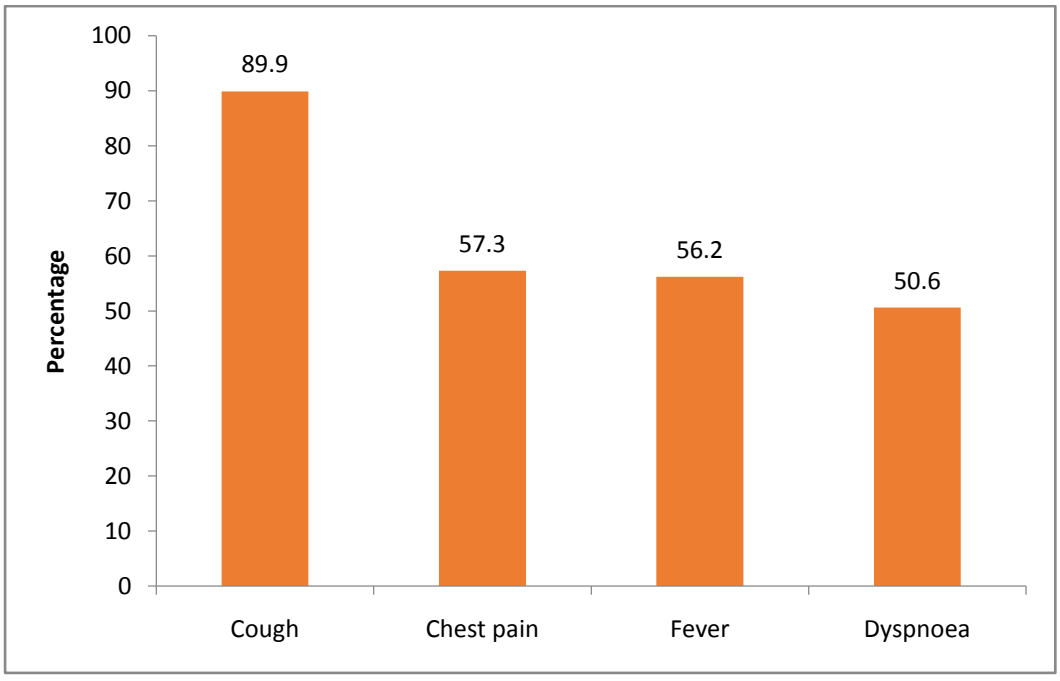

Figure 4: Distribution of suspected patients of Pulmonary aspergillosis on the basis of clinical symptoms (n=89)

Table 4: Chest X-ray findings in suspected patients of pulmonary aspergillosis (n=89)

Chest X-ray findings	Total (%)
Homogenous Haziness	36(40.4)
Homogenous Haziness + Consolidation	21(23.5)
Cavitary lesions + Consolidation	11(12.4)
No abnormality detected	11(12.4)
Cavitary lesions	10(11.2)

Out of 89 suspected cases, most common chest X-ray finding was Homogenous Haziness (40.4%).

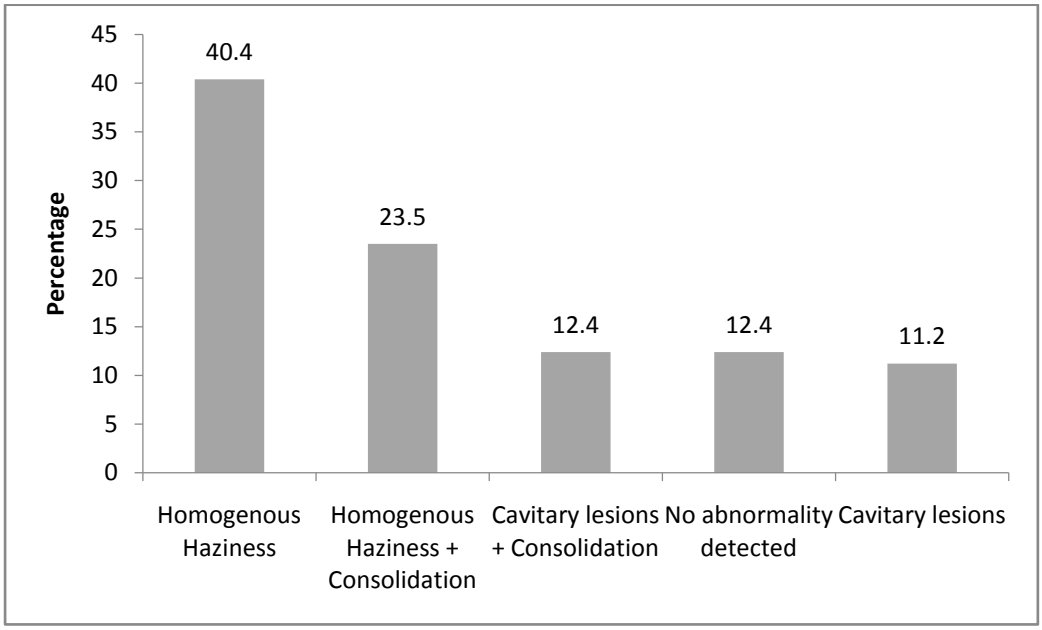

Figure 5: Chest X-ray findings in suspected patients of pulmonary aspergillosis (n=89)

Histopathological examination

Histopathological examination was done in 2 patients, but was not suggestive of Pulmonary Aspergillosis.

MYCOLOGICAL PROFILE

Table 5: Findings on KOH mount (n=89)

KOH mount	Total (%)
Hyaline septate fungal hyphae	49 (55.1)
No fungal elements seen	40 (44.9)

On KOH mount examination, hyaline septate fungal hyphae were found in 55.1%. However, 44.9% of the samples tested did not reveal any fungal element.

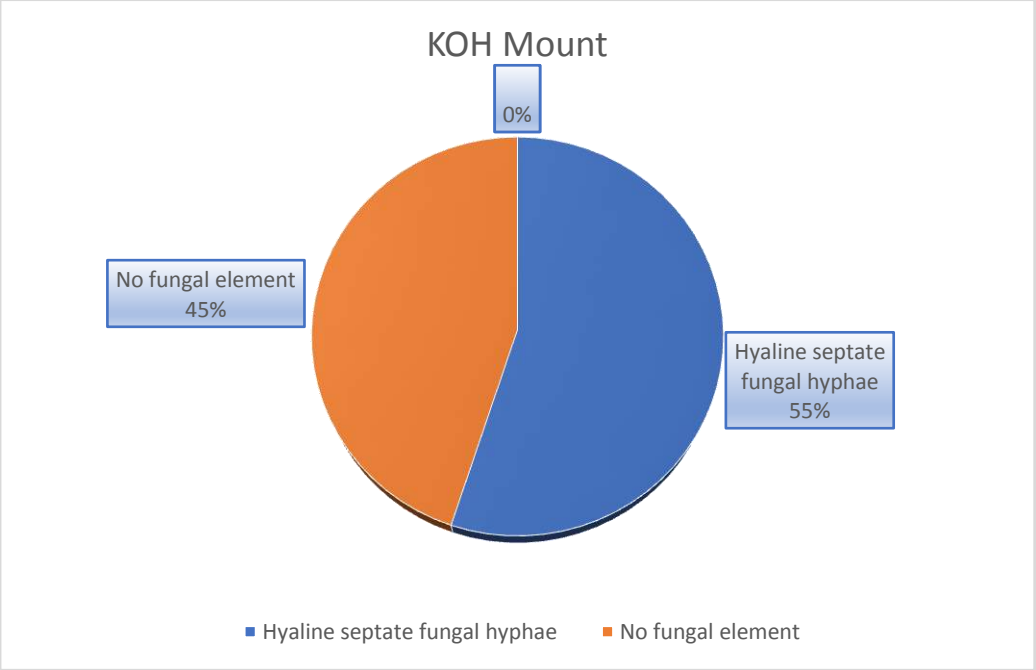

Figure 6: Findings on KOH mount (n=89)

Table 6: Findings on Fungal culture (n=89)

Culture	Total (%)
Growth	55 (61.8)
No growth	34 (38.2)

Out of 89 samples subjected to fungal culture, 55(61.8%) showed fungal growth while 34(38.2%) showed no growth

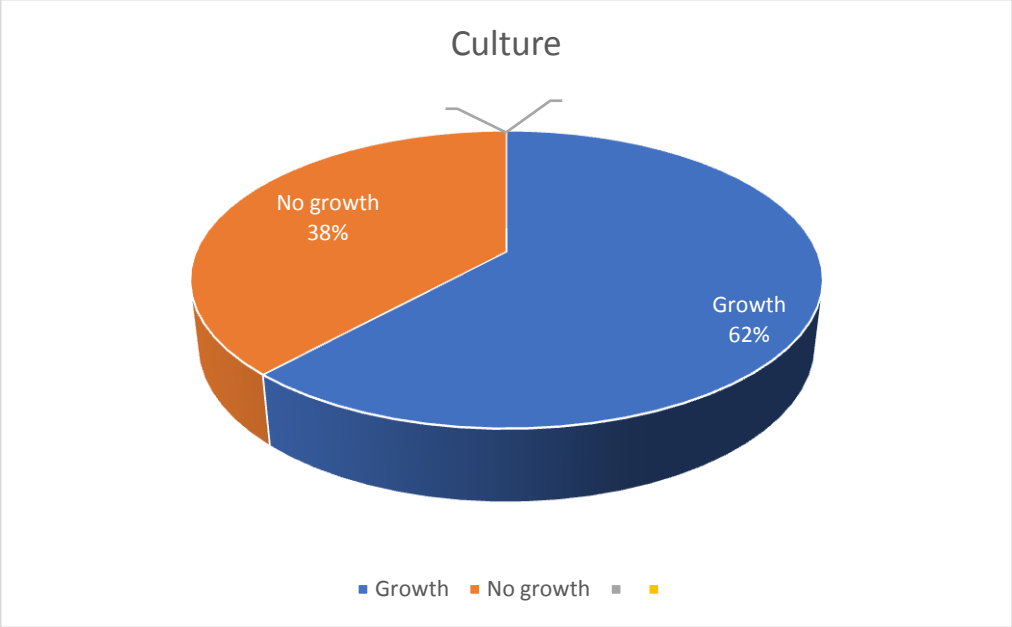

Figure 7: Distribution of Culture findings (n=89)

Table 7: Distribution of *Aspergillus* species (n=55)

***Aspergillus* species**	**Total (%)**
Aspergillus flavus	42 (76.4)
Aspergillus fumigatus	13 (23.6)

Out of 55 *Aspergillus* species most common species isolated was *A. flavus* (76.4%)followed by *A. fumigatus* (23.6%).

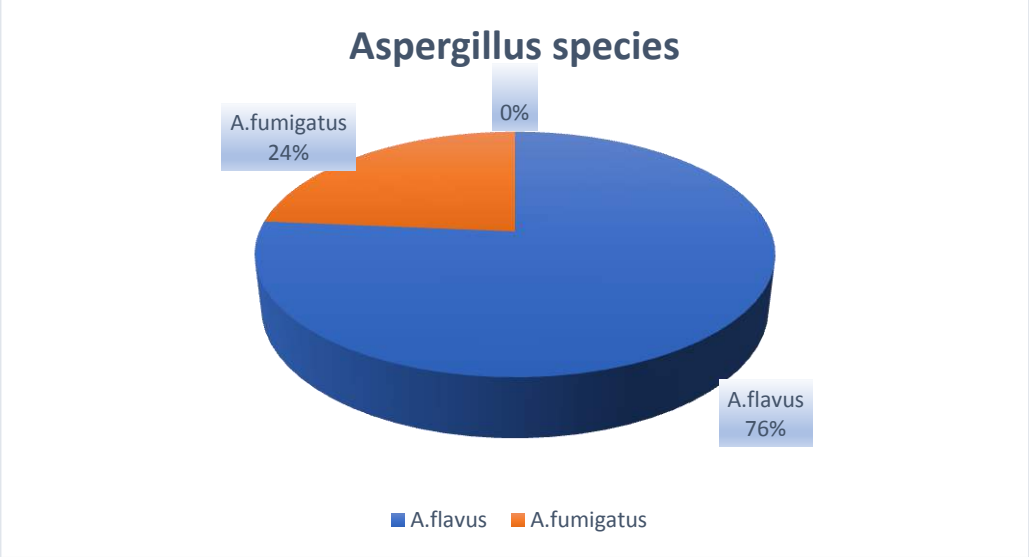

Figure 8: Distribution of *Aspergillus* species (n=55)

Table 8: Correlation of fungal smear with fungal culture (n=89)

	Fungal culture Positive (n=55)	**Fungal culture Negative (n=34)**
Fungal smear positive (n=49)	35 (39.3)	14 (15.7)
Fungal smear negative (n=40)	20 (22.5)	20 (22.5)

Out of 89 patients with suspicion of pulmonary aspergillosis, 39.3% of patients were positive both by fungal smear and culture. While 22.5% had positive fungal culture with negative fungal smear and 15.7% of patients had only positive fungal smear.69 (77.5%) patients have evidence of mycological criteria and 20(22.5%) patients had no mycological evidence.

SEROLOGICAL PROFILE

- Galactomannan test was done in 50 patients.
- (1-3)-β-D glucan assay was done in 30 patients.
- IgE was done in 14 patients. Among that 14 patients, 4 patients showed IgE levels of >1000 IU/ml.

Table 9: Galactomannan assay in suspected patients of pulmonary aspergillosis (n=50)

Galactomannan assay	Total (%)
Positive	28 (56)
Negative	22 (44)

Galactomannan assay positivity among suspected patients of pulmonary aspergillosis was 56 %.

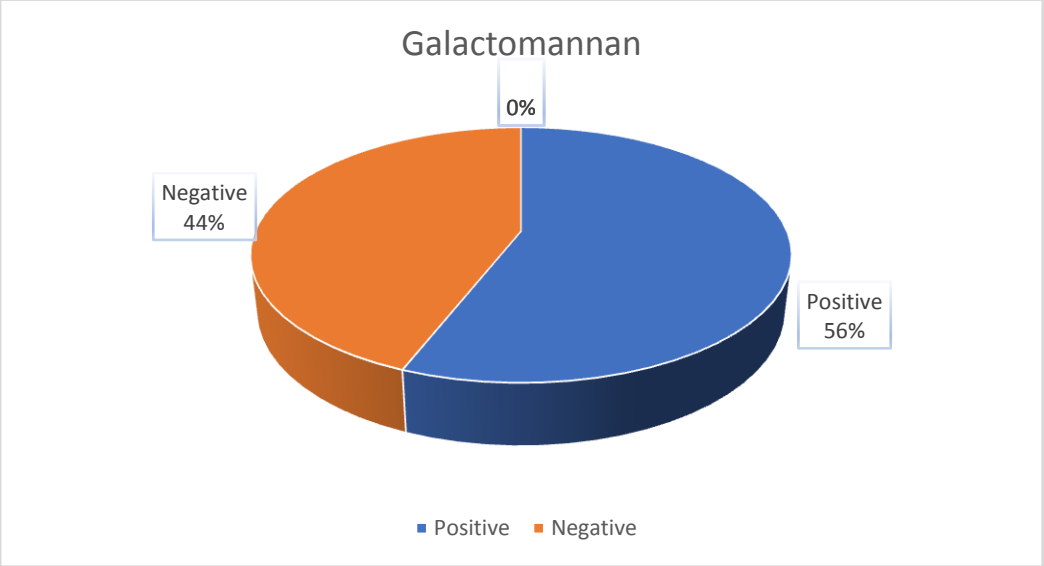

Figure 9: Positivity of galactomannan assay in suspected patients of pulmonary aspergillosis (n=50)

Table 10: (1-3)-β-D glucan assayin suspected patients of pulmonary aspergillosis (n=30)

(1-3)-β-D glucan assay	Total (%)
Positive	25 (83.3%)
Negative	5 (16.7%)

(1-3)-β-D glucan assaypositivity was seen in 83.3% cases.Single case had only fungitell positive but was galactomannan negative. So that could be due to other fungal infection.

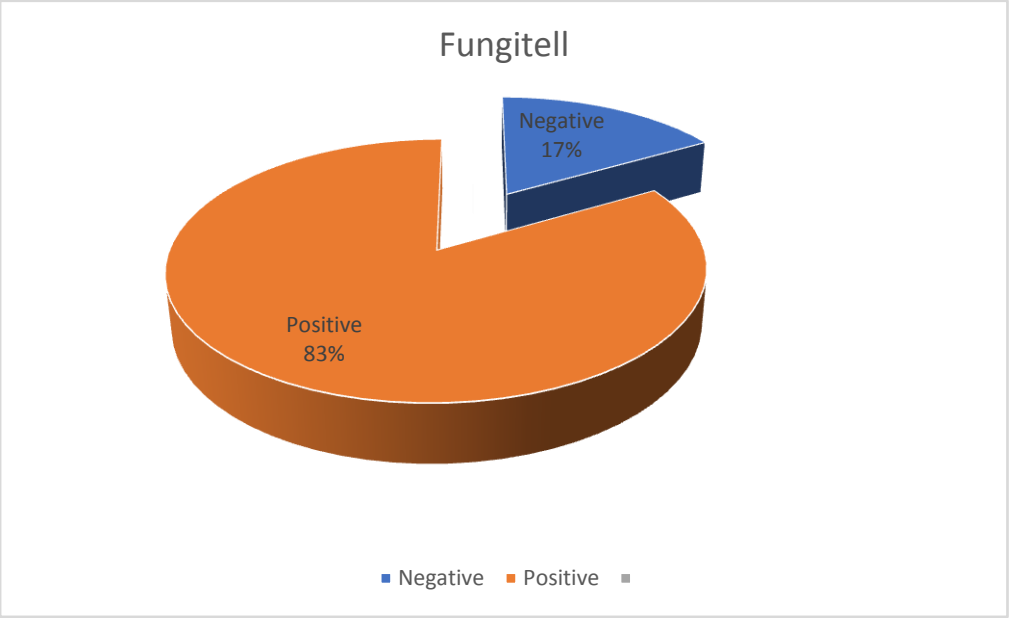

Figure 10: Positivity of (1-3)-β-D glucan assay in suspected patients of pulmonary aspergillosis (n=30)

Aspergillus skin test (Intradermal test)

No request was received for this test. So findings of this test could not be correlated.

Table 11: Correlation of mycological profile and serological profile (Galactomannan) in suspected patients of pulmonary aspergillosis (n=50)

Galactomannan assay	GM assay Positive (n=28)	GM assay Negative (n=22)
Mycological profile positive	28 (100%)	18 (81.8%)
Mycological profile Negative	0 (0%)	4 (18.2%)

Out of 50 patients which were tested for galactomannan assay, 100% patients were positive both by serological and mycological evidence.

Statistic	Value	95% CI
Sensitivity	100.00%	87.66% to 100.00%
Specificity	18.2%	5.19% to 40.28%
Positive Likelihood Ratio	1.22	1.00 to 1.49
Negative Likelihood Ratio	0	
Disease prevalence (*)	56.00%	41.25% to 70.01%
Positive Predictive Value (*)	60.87%	56.09% to 65.45%
Negative Predictive Value (*)	100.00%	
Accuracy (*)	64.00%	49.19% to 77.08%

Table 12: Correlation of mycological profile and serological profile((1-3)-β-D glucan assay) in suspected patients of pulmonary aspergillosis (n=30)

(1-3)-β-D glucan assay	(1-3)-β-D glucan assay Positive (n=25)	(1-3)-β-D glucan assay Negative (n=5)
Mycological profile positive	24 (96%)	5 (100%)
Mycological profile Negative	1 (4%)	0(0%)

Out of 30 patients who were tested for (1-3)-β-D glucan assay, 96% of patients were positive both by serological and mycological evidence. While 4% had positive serological evidence with negative mycological evidence.

Statistic	Value	95% CI
Sensitivity	96.00%	79.65% to 99.90%
Specificity	0.00%	0.00% to 52.18%
Positive Likelihood Ratio	0.96	0.89 to 1.04
Negative Likelihood Ratio		
Disease prevalence (*)	83.33%	65.28% to 94.36%
Positive Predictive Value (*)	82.76%	81.59% to 83.87%
Negative Predictive Value (*)	0	
Accuracy (*)	80.00%	61.43% to 92.29%

CHARACTERIZATION OF PATIENTS WITH SUSPECTED PULMONARY ASPERGILLOSIS

- Out of total 89 suspected cases of pulmonary aspergillosis,
- No radiological, mycological and serological evidence of fungal infection was observed among 6 patients.
- 4 had only mycological evidence but no radiological and serological evidence. So, these fungal infections could be considered as colonisers.
- On the basis of raised IgE levels, radiological, mycological evidence but no serological evidence of invasive aspergillosis, 4 cases were labelled as cases of Allergic Bronchopulmonary Aspergillosis (ABPA).
- 75 cases, were labelled as cases of Invasive Aspergillosis (IA) on the basis of Host, Clinical and Mycological criteria.

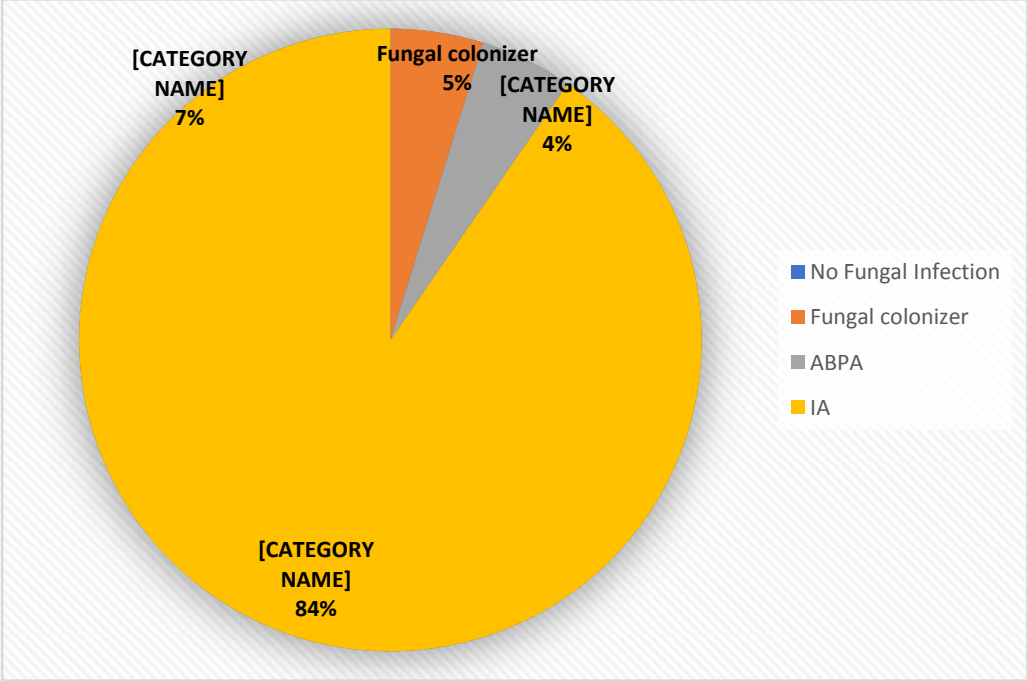

Figure 11: Characterisation of suspected patients of pulmonary aspergillosis (n=89)

Table 13: Characterisation of patients of InvasiveAspergillosis (n=75)

	Number(%)
POSSIBLE	75 (84.3%)
Host+Clinical	
PROBABLE	69 (77.5%)
Host+Clinical+Mycological	
PROVEN	0 (0%)
Host+Clinical+ Mycological+ HPE	

Table 14: Outcome of the patients. (n=89)

OUTCOME	No. OF CASES	PERCENTAGE
DISCHARGED	70	78.6%
EXPIRED	19	21.4

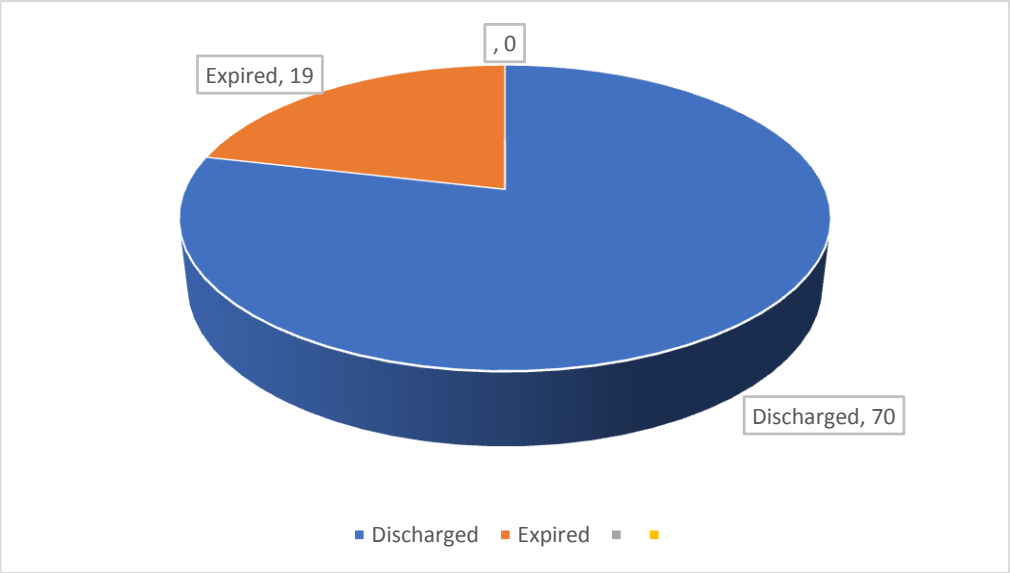

Figure 12: Outcome of patients. (n=89)

Table 15: Comparison of various variables affecting outcome in suspected patients of Invasive Aspergillosis (n=89)

		Outcome				p-value
		Discharged		Expired		
Age (Mean/sd)		58.01	13.69	61.68	13.75	**0.303**
Sex	F	30	42.86%	5	26.32%	0.191
	M	40	57.14%	14	73.68%	
Presenting Complaints						
Fever	N	32	45.71%	7	36.84%	0.605
	Y	38	54.29%	12	63.16%	
Cough	N	9	12.86%	0	0.00%	0.195
	Y	61	87.14%	19	100.00%	
Chest pain	N	31	44.29%	7	36.84%	0.611
	Y	39	55.71%	12	63.16%	
Dyspnea	N	35	50.00%	9	47.37%	0.839
	Y	35	50.00%	10	52.63%	
Host factors						
Malignancy	N	67	95.71%	19	100.00%	0.359
	Y	3	4.29%	0	0.00%	
Solid organ transplant	N	68	97.14%	19	100.00%	0.456
	Y	2	2.86%	0	0.00%	
HIV	N	68	97.14%	18	94.74%	0.518
	Y	2	2.86%	1	5.26%	
DM	N	35	50.00%	9	47.37%	0.839
	Y	35	50.00%	10	52.63%	
Steroid therapy	N	42	60.00%	11	57.89%	0.868
	Y	28	40.00%	8	42.11%	
Clinical criteria-Chest X-ray	NAD	11	15.71%	0	0.00%	0.111
	y	59	84.29%	19	100.00%	
Mycological evidence	N	17	24.29%	3	15.79%	0.431
	Y	53	75.71%	16	84.21%	
Serological evidence						
Galactomannan assay (Mean/sd)		0.87	0.86	1.22	1.39	0.278
(1-3)-β-D glucan assay (Mean/sd)		197.46	126.61	353.91	211.47	0.017
IgE levels (Mean/sd)		708.49	605.61	3408.5	4643	0.153
Characterisation	Fungal infection	60	85.71%	19	100.00%	0.0001
	Colonizers	4	5.71%	0	0.00%	
	NO F.I.	6	8.57%	0	0.00%	

DISCUSSION

This prospective study was conducted in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. A total of 89 consecutive cases of suspected invasive aspergillosis were included in the study. The clinical and microbiological profiles of suspected cases of pulmonary aspergillosis were studied.

In the present study of 89 consecutive cases of suspected invasive aspergillosis, the mean age of the patients was 58.7 (18 years to 89 years). The similar observation was seen in the study of Khorvash *et al* in which mean age of the patients was found to be 54.95 ± 18.82 (range 20 yrs to 86 yrs)[63].

In the present study, suspected invasive aspergillosis was more common in males (60.6%) as compare to females (39.3%). This can be explain by the fact that the males are more exposed to fungal spores during working with the decaying vegetation like mold hay in agriculture. These findings were more accordant with the study conducted by Cordonnier et al [64] and S. Khanna et al [65]**,** in which male predominance was reported.

Most common risk factor among suspected patients of pulmonary aspergillosis was diabetes (50.6%) followed by chronic steroid use (40.4%).These results were in concordance with the study conducted by Kurhade et al [3]. But these results were disparate from the study of Herbrecht et al, in which hematological malignancies (63.2%) was seen as the most common risk factor followed by hematopoietic stem cell transplantations (21.3%)[66].

Most common presentation was cough (89.9%) followed by chest pain (57.3%) and fever (56.2%). In contrast to the study of N. Tutar [67] in which the most common symptom was dysnoea followed by fever and cough.

In our study, most common chest X-ray finding was Homogenous Haziness (40.4%). Similar findings were observed in study conducted by Nancy et al[26].

Galactomannan assay positivity among suspected patients of pulmonary aspergillosis was 56 %. In contrast to the study of Maertens et al who reported the positivity of GM-ELISA only 8.9% [51].

In present study, positive fungal smear in patients with suspicion of invasive aspergillosis was present in 55.1% while fungal culture positivity for *Aspergillus sp.* was present in 61.8% patients. In contrast to the study conducted by Cordonnier et al, patients with positive fungal smear was seen in 6.6% and culture in 71.4% patients.[64]

A.flavus (76.4%) was found to be most common species isolated followed by *A.fumigatus* (23.6%) in our study. Similar observation was seen in the study by I. Xess et al, in which most common species isolated was *A.flavus* (46.9%) followed by *A.fumigatus* (37.7%)[51]. These findings were not accordant with the study conducted by M. Subira which showed that most common *Aspergillus* spp. isolated was *A. fumigatus* (41%)[67].

In our study, suspicion of Pulmonary Aspergillosis were categorized, ABPA (4.5%), Fungal colonizers (4.5%), other fungal infections (7%) and Invasive Aspergillosis (84%). Cases of IA were further classified as proven IA (0%), probable IA (77.5%) and possible IA (84.3%) as per European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria. In a study conducted by Khanna et al, 81 patients were categorized as proven IA (14.8%), probable IA (48.2%), possible IA (27.2%), or no IA (8, 9.9%) as per EORTC/MSG criteria[65].

Few studies have been performed for the direct comparisons of the 2 assays for the diagnosis of IA in the same patients. Some favored the BG assay, some favoured the GM assay and some have no major differences between the two assays[68]. In the present study of 89 consecutive cases of suspected invasive aspergillosis, 18 cases (22%) were positive both by galactomannan and fungitell.

According to literature the proportion of patients with IA that are positive for GEI continues to vary ranging from 13% to 87.5%. The specificity of the assay on serum tends to range from 21% to 100% and in most cases was >80% [69].This was similar to our study in which senstivity came out to be 100% and specificity 18.2%. Almost similar findings were seen in the study conducted by Okuturlar et al., 2015, in which sensitivity was 100% [70]. In contrast to the studies of Kimura et al., 2009 [71] and Hsu et al., 2010 [72] in which sensitivity was 83.3% and specificity was 81.1% and 71.2% respectively.

Out of 30 patients who were tested for (1-3)-β-D glucan assay, 80% of patients were positive both by serological and mycological evidence. While 3.3% had positive serological evidence with negative mycological evidence. The sensitivity came out to be 82.76%. In contrast to the study in which BG tests were positive in 85.7% of patients with proven and 52.4% of patients with probable aspergillosis[73].

Mortality rate in suspected patients of invasive aspergillosis was 21.4% in our study. In a study conducted by N. Tutar et al, mortality was seen in 54.5% patients[74]. High mortality rate of 30% to 70% was seen in some studies [75,76].

In present study, variables associated with poor outcome in patients with suspicion of pulmonary aspergillosis were positive fungal culture, positive galactomannan and fungitell, higher age group and poor host factors. Histopathological examination was not done in all patients so this leads to the failure of meeting proven criteria of Invasive Aspergillosis.

SUMMARY AND CONCLUSION

SUMMARY:

- A total of 89 consecutive cases of suspected invasive aspergillosis were included in the study. The clinical and microbiological profiles of suspected cases of pulmonary aspergillosis were studied.
- The mean age group affected was 58.7 yrs.
- Majority were males (60.6%) as compared to females (39.3%).
- Most common risk factor among suspected patients of pulmonary aspergillosis was diabetes (50.6%) followed by chronic steroid use (40.4%).
- Most common presentation was cough (89.9%) followed by chest pain (57.3%) and fever (56.2%).
- Out of 89 suspected cases, most common chest X-ray finding was Homogenous Haziness (40.4%).
- On KOH mount examination, hyaline septate fungal hyphae were found in 55.1%. However, 44.9% of the samples tested did not reveal any fungal element.
- Out of 89 samples subjected to fungal culture, 55 (61.8%) showed fungal growth while 34 (38.2%) showed no growth.
- 77.5% patients had evidence of fungal infection by smear/culture.
- Out of 55 *Aspergillus* species most common species isolated was *A. flavus* (76.4%)followed by *A. fumigatus* (23.6%).
- IgE was done in 14 patients& 4 patients showed IgE levels of >1000 IU/ml.
- Galactomannan assay positivity among suspected patients of suspected pulmonary aspergillosis was 56 %.
- (1-3)-β-D glucan assay positivity among suspected patients of pulmonary aspergillosis was 83.3 %.
- No request was received for Aspergillus skin test.
- Histopathological examination, being an invasive procedure could not be done in all patients

- Out of 50 patients which were tested for galactomannan assay, 100% patients were positive both by serological and mycological evidence. The sensitivity and specificity of the test came out to be 100% and 18.2% respectively.
- Out of 30 patients who were tested for (1-3)-β-D glucan assay, 96% of patients were positive both by serological and mycological evidence. The sensitivity of this test was 96%.
- In 6 cases no evidence of fungal infection was observed.
- 4 cases were labelled as cases of Allergic Bronchopulmonary Aspergillosis (ABPA) on the basis of raised IgE levels.
- 4 had only mycological evidence but no radiological and serological evidence and they were considered as colonisers.
- 75 cases, were labelled as cases of Invasive Aspergillosis (IA) on the basis of Host, Clinical and Mycological criteria.
- 75 cases of Invasive Aspergillosis were further categorized on the basis of EORTC/MSG criteria as Possible IA (100%), Probable IA (92%) and Proven IA (0%).
- The mortality in suspected patients of invasive aspergillosis was observed in 21.4% patients.
- Variable which mainly affected the results of our study was fungal infection among the patients.
- In present study, variables associated with poor outcome in patients with suspicion of pulmonary aspergillosis were positive fungal culture, positive galactomannan and fungitell, higher age group and poor host factors.

CONCLUSION:

In this prospective study conducted over period of 1 year, a total of 89 consecutive cases with clinical suspicion of pulmonary aspergillosis admitted to DMC&H were included in the study. Delayed diagnosis may lead to poor outcomes. The study demonstrated that galactomannan assay and (1-3)-β-D glucan assay are useful diagnostic modality with a high sensitivity and specificity for the detection of

pulmonary aspergillosis in high risk patients along with positive fungal culture. Thus, these tests are early markers for invasive aspergillosis.

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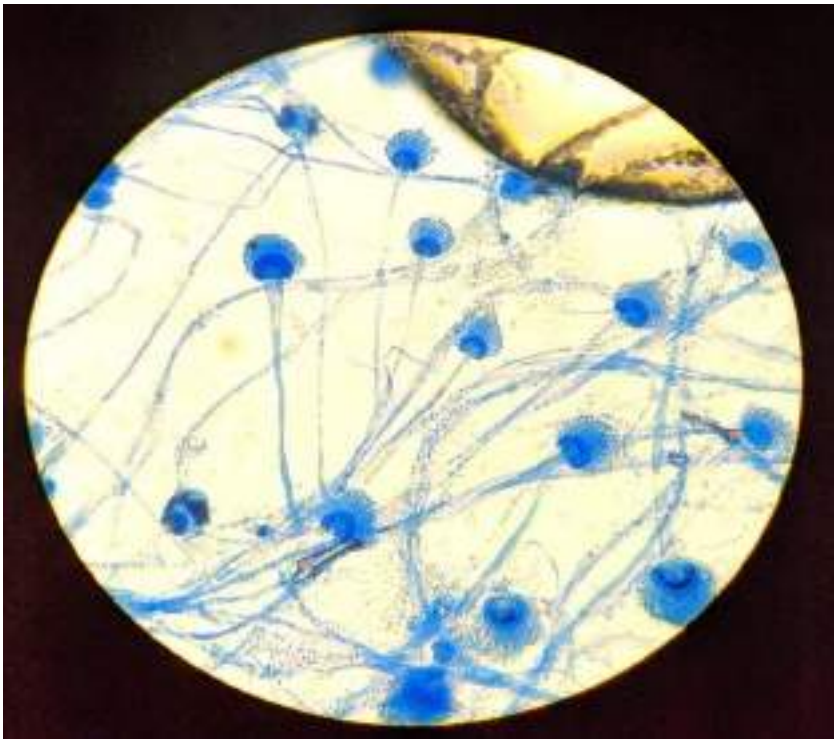
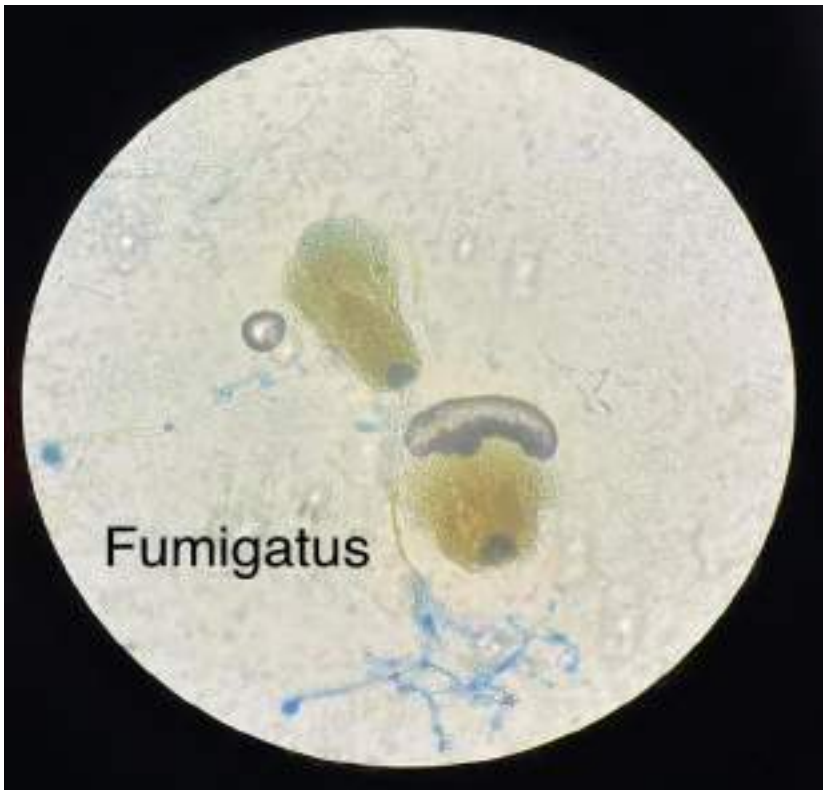
Picture 1: Septate hyphae

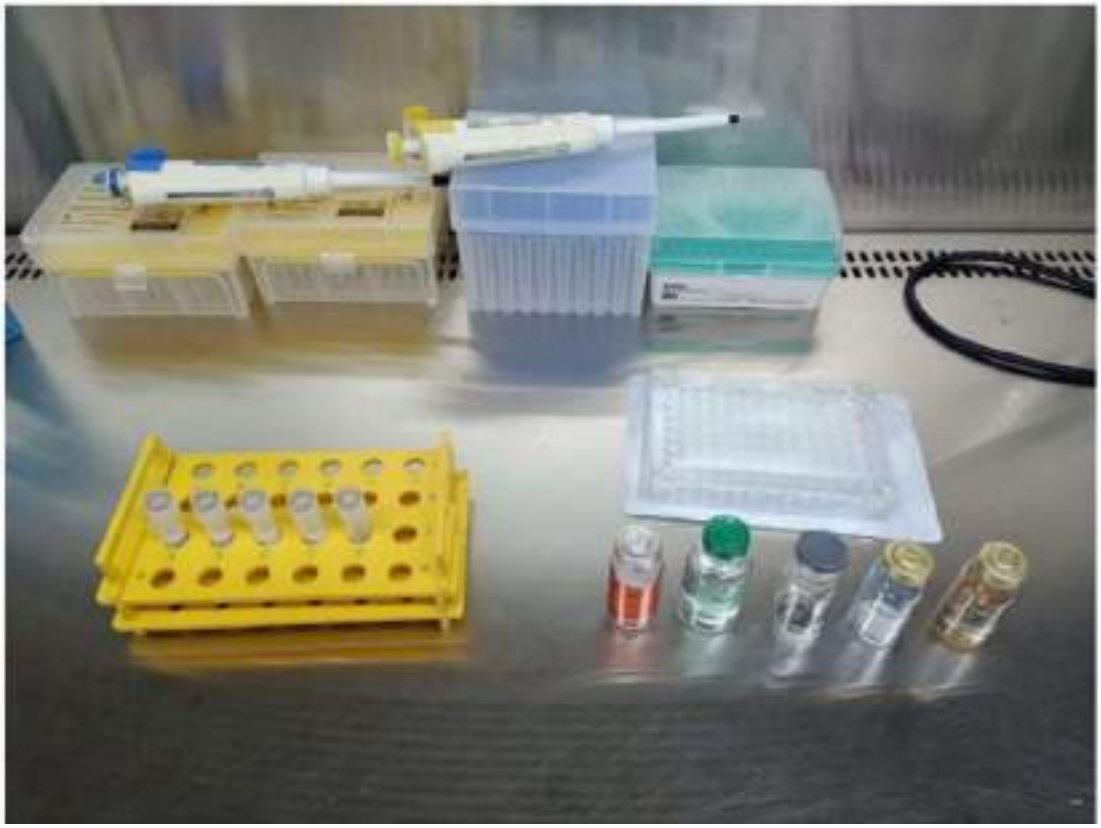


Picture 2: ***A. flavus***

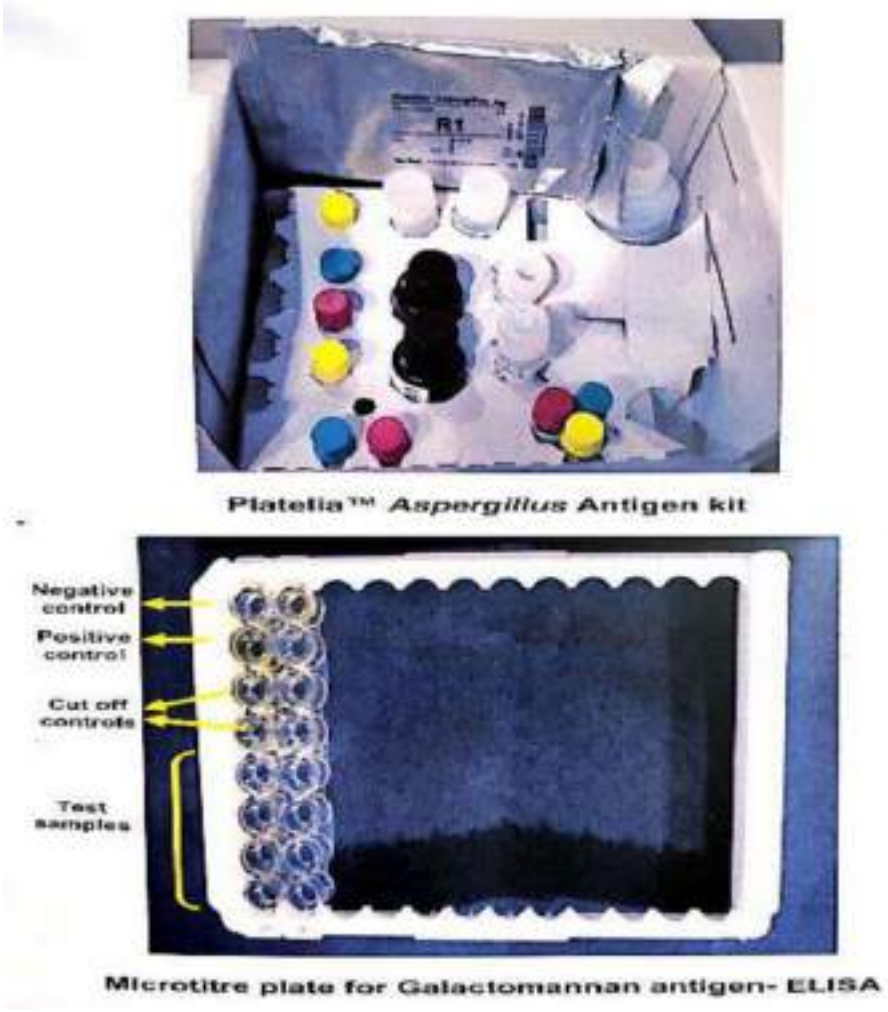


Picture 3: ***A. fumigatus***

Picture 4: Fungitell ((1-3)-beta-D-glucan) kit



Picture 5: Standards, Reagents, ELISA tray



Picture 6: Microtitre plate fpr Galactomannan antigen-ELISA

Picture 7: Kinetic reader

KEY TO MASTER CHART

M	Male
F	Female
Y	Yes
N	No
NAD	No abnormality detected

S.No	Name	Age	Sex	MRD No.	Lab No.	Specimen	Hb	TLC	Eosinophil count	Galactomannan assay	(1-3)-β-D glucan assay	IgE levels	Fungal smear	Fungal culture	Fever	Cough	Chest pain
1	JLM	67	F	231396	36	Sputum	9.8	10.9	0.1	0.29		19.42	Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
2	PKK	58	M	876231	64	Sputum	12.3						No fungal elements seen	No growth	Y	N	N
3	BKT	55	M	439771	101	ET	11						No fungal elements seen	No growth	N	Y	N
4	VV	86	F	608504	150	Sputum	9.4	11.2	0.2	0.65		1404	Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
5	SB	34	F	878423	162	ET	8.1	12.3					No fungal elements seen	No growth	N	Y	Y
6	GK	86	F	879484	180	ET	10.8	8.4	0.2	1.83			Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	Y
7	RL	70	M	665300	198	Sputum	11.1	16.3	1.8	0.06			Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
8	RK	51	M	882132	281	ET	10.4	21.7					No fungal elements seen	No growth	N	Y	Y
9	PS	58	M	883320	303	ET	12.1	10.3	0	2.25			Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	Y
10	KJ	72	F	149990	348	Sputum	11.4	10.8	0.1	0.11	56.08		Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	Y
11	GK	53	F	24970	352	Sputum	7.7		6.6				No fungal elements seen	No growth	N	N	Y
12	AS	32	M	886544	353	ET	10.6	19.7					No fungal elements seen	No growth	Y	N	Y
13	GA	27	M	886487	361	ET	11.3		9.7				No fungal elements seen	No growth	Y	Y	N
14	CS	60	M	881627	54	ET	12.4	26.6	0	1.71	138.69	1081	Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	Y
15	JS	46	M	796826	78	ET	10.9	10	0	1.57	300.91		No fungal elements seen	Aspergillus fumigatus	N	Y	Y
16	PD	65	F	889474	440	Sputum	10.4	10.7					No fungal elements seen	No growth	N	Y	N
17	PKK	67	M	882742	504	Sputum	8.2						No fungal elements seen	No growth	Y	Y	N
18	NK	59	F	133984	593	ET	8.3	19.8					No fungal elements seen	No growth	N	Y	N
19	DS	72	M	893380	594	Sputum	10	15.3					No fungal elements seen	No growth	Y	Y	N
20	JK	25	F	893215	754	ET	5.8	1.3					Hyaline septate fungal hyphae	Aspergillus flavus	N	Y	Y
21	SL	64	F	903181	763	Sputum	11.7	21.3				305.43	Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
22	MK	55	F	905806	784	ET	13	12.9					No fungal elements seen	No growth	N	N	N
23	PL	60	F	905776	321	Sputum	6.4	26.3					No fungal elements seen	No growth	N	Y	N
24	DC	74	F	892133	110	ET	10.5						Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
25	PA	72	M	894111	138	Sputum	8.4	13.9	3.5	0.82	77.04		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
26	SN	62	M	898873	152	Sputum	9.8			0		1413	Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
27	RC	66	M	896413	155	ET	11.2	12.6		0.45	78.82	1692	No fungal elements seen	Aspergillus flavus	N	Y	Y
28	SA	57	F	902844	165	ET	10.6			0.07			No fungal elements seen	No growth	N	Y	N
29	KT	64	M	900494	182	Sputum	13	20.2		1.56		1415	No fungal elements seen	Aspergillus flavus	Y	Y	Y
30	HK	53	F	402953	191	Sputum	9.1	13.5					No fungal elements seen	Aspergillus flavus	Y	N	Y
31	AM	47	F	904870		Sputum	9.3		1.9	0.06			No fungal elements seen	No growth	N	Y	N
32	RR	66	F	906721		Sputum							Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
33	JLM	62	M	909472		ET	8.7		0.1	4.71	523		Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	N
34	S	31	F	782280		Sputum	6.7		12.3	0.1			No fungal elements seen	No growth	N	Y	N
35	BK	51	F	169640		Sputum	10.6		0			690.4	No fungal elements seen	No growth	Y	Y	N
36	KS	63	M	908647		Sputum	10.1	23.5	1.6	0.28	161		No fungal elements seen	No growth	N	Y	N
37	TR	60	M	910213		ET	9.4	15.8	0				Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
38	PC	58	M	916839		Sputum							No fungal elements seen	No growth	N	Y	N
39	DR	75	M	844642	226	Sputum				0.09		90.39	Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
40	SK	27	F	916169	274	ET							No fungal elements seen	Aspergillus flavus	N	Y	Y
41	DRB	72	M	914749	298	Sputum				0.16			Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
42	SK	73	F	920426	310	Sputum				0.92	213.66		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
43	D	18	M	922958		Sputum	13.6	14.7	17.5	0.14		9430	Hyaline septate fungal hyphae	No growth	Y	Y	N
44	NL	60	M	923678		Sputum	10.3	17.7					Hyaline septate fungal hyphae	No growth	N	Y	N
45	AS	67	M	924498		ET	10.2						Hyaline septate fungal hyphae	No growth	N	Y	Y

S.No	Name	Dyspnea	Suspected pulmonary infection	Malignancy	Solid organ transplant	HIV	HTN	DM	Steroid therapy	CXR	CXR	Outcome	Characterization	IA
1	JLM	Y	N	N	N	N	N	Y	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
2	PKK	N	N	N	N	N	Y	N	N	NAD		D	NO F.I.	
3	BKT	N	N	N	N	N	Y	N	N	NAD		D	NO F.I.	
4	VV	Y	Y	N	N	N	Y	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
5	SB	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	
6	GK	Y	N	N	N	N	Y	Y	N	Homogenous haziness		D	IA-Probable	Possible
7	RL	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
8	RK	N	N	N	N	N	N	Y	N	NAD		D	NO F.I.	
9	PS	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
10	KJ	Y	N	N	N	N	N	N	N	Homogenous haziness		E	IA-Probable	Possible
11	GK	N	N	N	N	N	Y	Y	N	Homogenous haziness		D	IA-Probable	Possible
12	AS	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
13	GA	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
14	CS	N	Y	N	N	N	Y	N	Y	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
15	JS	Y	N	N	N	N	N	N	Y	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
16	PD	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
17	PKK	N	N	N	N	N	Y	N	N	Homogenous haziness		E	IA-Probable	Possible
18	NK	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
19	DS	N	N	N	N	N	N	N	N	Homogenous haziness		E	IA-Probable	Possible
20	JK	Y	N	N	N	N	N	N	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
21	SL	Y	Y	N	N	N	Y	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
22	MK	Y	N	N	N	N	N	N	N	NAD		D	NO F.I.	
23	PL	N	N	N	N	N	N	N	N	Homogenous haziness		E	IA-Probable	
24	DC	N	N	N	N	N	N	Y	N	Homogenous haziness		D	IA-Probable	Possible
25	PA	Y	N	N	N	N	Y	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
26	SN	Y	Y	N	N	N	N	Y	N	Homogenous haziness	Consolidation	E	ABPA	
27	RC	Y	Y	N	N	N	N	Y	Y	Cavitatory lesions	Consolidation	E	ABPA	
28	SA	N	N	N	N	N	N	Y	Y	Homogenous haziness		D	IA-Probable	
29	KT	Y	Y	N	N	N	N	N	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
30	HK	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
31	AM	N	N	N	N	N	Y	Y	Y	Homogenous haziness		D	IA-Probable	
32	RR	Y	N	N	N	N	N	Y	Y	Homogenous haziness		D	IA-Probable	Possible
33	JLM	Y	N	N	N	N	N	N	Y	Cavitatory lesions	Consolidation	E	IA-Probable	Possible
34	S	N	N	N	N	N	N	N	Y	Homogenous haziness		D	IA-Probable	
35	BK	N	Y	N	N	N	Y	Y	N	NAD		D	NO F.I.	
36	KS	N	N	N	N	N	N	Y	Y	Homogenous haziness		D	IA-Probable	Possible
37	TR	Y	N	N	N	N	N	Y	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
38	PC	N	N	N	N	N	N	Y	Y	NAD		D	NO F.I.	
39	DR	Y	Y	N	N	N	Y	Y	Y	Homogenous haziness		E	IA-Probable	Possible
40	SK	Y	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
41	DRB	N	N	N	N	N	N	N	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
42	SK	Y	Y	N	N	N	N	N	N	Cavitatory lesions		D	IA-Probable	Possible
43	D	N	Y	N	N	N	N	N	Y	Homogenous haziness	Consolidation	E	ABPA	
44	NL	N	N	N	N	N	N	Y	N	NAD		D	Colonizers	
45	AS	N	N	N	N	N	N	Y	N	NAD		D	Colonizers	

S.No	Name	Age	Sex	MRD No.	Lab No.	Specimen	Hb	TLC	Eosinophil count	Galactomannan assay	(1-3)-β-D glucan assay	IgE levels	Fungal smear	Fungal culture	Fever	Cough	Chest pain
46	SG	74	F	933106		Sputum	8.6		5.9	0.08		44.16	Hyaline septate fungal hyphae	No growth	Y	Y	N
47	JG	75	M	889917		Sputum	8.6		7.6	0.1	84.04		Hyaline septate fungal hyphae	No growth	N	Y	Y
48	JR	66	M	61587		Sputum	7	12.5		1.44	523		Hyaline septate fungal hyphae	No growth	N	Y	Y
49	SS	52	M	1096713	903	ET	13	19.5		0.64	121.96		Hyaline septate fungal hyphae	No growth	Y	Y	N
50	JS	60	M	1048362	968	Sputun	7.9		8.3				Hyaline septate fungal hyphae	No growth	Y	Y	N
51	SC	68	M	1113723	1310	ET	13.7	13.9	1.1	2.87	523		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
52	SS	79	M	1118299	1389	ET	10.5	4.3	5	2.88			Hyaline septate fungal hyphae	No growth	N	Y	N
53	KS	79	M	1118841	1465	ET	11.7	19.6	0.1	0.81	523		Hyaline septate fungal hyphae	No growth	N	Y	Y
54	SS	75	M	1119045	1466	ET	11.8	32.9	0.5	1.61	156.57		Hyaline septate fungal hyphae	No growth	Y	Y	N
55	RV	32	M	1122850	1706	ET	13.5	8.3	0.4	0.41	79.4		Hyaline septate fungal hyphae	No growth	N	Y	N
56	KK	64	F	1135778	1930	ET	13.1	31.9	0.2	0.33	209.43		Hyaline septate fungal hyphae	No growth	Y	N	N
57	RS	66	M	1134066	1970	Sputum	9.8	15.6	0.7				Hyaline septate fungal hyphae	Aspergillus flavus	N	Y	N
58	BS	56	M	1134612	1972	Sputum	14.6	17.6	0	1.09			Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	N
59	PT	35	M	1138757	1998	ET	9.7	3.6	0.6				Hyaline septate fungal hyphae	Aspergillus flavus	Y	N	N
60	SS	58	M	1054132	134	ET	9.3	38.9					No fungal elements seen	Aspergillus flavus	N	Y	Y
61	PS	60	M	1060343	160	ET	10	19		0.66	142.05		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
62	KS	56	M	1050992	161	ET							No fungal elements seen	Aspergillus flavus	N	Y	Y
63	RS	73	M	994285	172	Sputum	11						No fungal elements seen	Aspergillus fumigatus	Y	Y	Y
64	CD	70	F	1108181	230	ET							Hyaline septate fungal hyphae	Aspergillus flavus	N	Y	Y
65	MK	52	F	1104694	231	ET				0.52			Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
66	PKK	57	M	1101439	281	Sputum				0.04			Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
67	SS	55	M	1110944	289	ET				2.03	372.38		Hyaline septate fungal hyphae	Aspergillus fumigatus	N	Y	Y
68	HK	69	M	1108207	292	ET				0.45	115.87		No fungal elements seen	Aspergillus flavus	Y	Y	N
69	SR	69	F	1112375	304	ET				1.69	523		No fungal elements seen	Aspergillus flavus	N	Y	Y
70	SB	54	F	1109251	309	ET				0.26	222.19		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
71	GK	65	F	1113930	310	Sputum				0.39	523		No fungal elements seen	Aspergillus flavus	N	Y	Y
72	GS	57	M	1115925	335	Sputum				3.19	382.33		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	Y
73	GS	52	M	545302	326	Sputum				0.32	114.66		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
74	HS	65	M	1119943	349	Sputum				0.73	193.65		No fungal elements seen	Aspergillus flavus	N	Y	Y
75	IS	54	M	1119818	357	Sputum				0.63	98.26		No fungal elements seen	Aspergillus flavus	N	N	Y
76	CK	65	F	1109250	377	Sputum				0.3			No fungal elements seen	Aspergillus flavus	N	Y	Y
77	RK	39	F	1124451	384	Sputum				0.61	196.86		No fungal elements seen	Aspergillus flavus	Y	Y	Y
78	SS	40	M	1125680	386	Sputum						10804	No fungal elements seen	Aspergillus flavus	Y	Y	Y
79	HS	50	M	1142443	2099	Sputum				3.46	421.14		Hyaline septate fungal hyphae	Aspergillus flavus	Y	N	Y
80	AS	58	M	1148631	2189	Sputum							Hyaline septate fungal hyphae	Aspergillus fumigatus	Y	Y	N
81	LJ	54	M	94653	424	Sputum							No fungal elements seen	Aspergillus flavus	N	Y	Y
82	SK	55	F	1163590	2491	ET	6.8	12.9	0				Hyaline septate fungal hyphae	No growth	Y	Y	N
83	KS	52	M	1148099	494	Sputum							No fungal elements seen	No growth	N	Y	N
84	BS	43	M	1154723	533	ET	12.5	13.5	2.6				No fungal elements seen	Aspergillus flavus	Y	Y	Y
85	LJ	77	F	499096	543	Sputum	11.3	8.1	1				Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
86	RK	61	F	914654	544	ET	8.2	25.2	0.4	0.57	46.89		Hyaline septate fungal hyphae	Aspergillus flavus	Y	Y	N
87	MD	73	F	920172	560	Sputum	13.3	11.6	3				No fungal elements seen	Aspergillus fumigatus	N	Y	Y
88	PL	59	M	395212	562	Sputum	7.4	10.6	3	0.95		385.8	No fungal elements seen	Aspergillus fumigatus	N	Y	Y
89	NS	60	F	1161090	568	Sputum	7.9	7.8	0	1.83	523	44.31	Hyaline septate fungal hyphae	Aspergillus fumigatus	N	Y	Y

S.No	Name	Dyspnea	Suspected pulmonary infection	Malignancy	Solid organ transplant	HIV	HTN	DM	Steroid therapy	CXR	CXR	Outcome	Characterization	IA
46	SG	N	Y	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
47	JG	N	N	N	N	N	Y	Y	Y	Homogenous haziness		D	IA-Probable	Possible
48	JR	N	N	Y	N	N	Y	Y	N	Cavitatory lesions		D	IA-Probable	Possible
49	SS	N	N	N	N	N	N	Y	N	Cavitatory lesions		E	IA-Probable	Possible
50	JS	N	N	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
51	SC	Y	N	N	N	N	Y	Y	Y	Cavitatory lesions		E	IA-Probable	Possible
52	SS	N	N	N	N	N	Y	N	N		Consolidation	D	IA-Probable	Possible
53	KS	N	Y	N	N	N	N	Y	N	Cavitatory lesions	Consolidation	E	IA-Probable	Possible
54	SS	N	Y	N	N	N	N	Y	N	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
55	RV	N	N	N	N	N	N	N	Y	Homogenous haziness		D	IA-Probable	Possible
56	KK	Y	N	N	N	Y	Y	N	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
57	RS	Y	N	Y	N	N	Y	N	Y	Homogenous haziness		D	IA-Probable	Possible
58	BS	Y	N	N	N	N	N	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
59	PT	Y	Y	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
60	SS	Y	N	N	N	N	Y	Y	Y	Homogenous haziness		D	IA-Probable	Possible
61	PS	Y	N	N	N	N	N	N	Y	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
62	KS	Y	N	N	N	N	Y	Y	N	Homogenous haziness		D	IA-Probable	Possible
63	RS	N	N	N	N	N	N	Y	N	Homogenous haziness		D	IA-Probable	Possible
64	CD	Y	Y	N	N	N	N	Y	N	Homogenous haziness	Consolidation	D	IA-Probable	Possible
65	MK	Y	N	N	N	N	N	N	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
66	PKK	N	Y	N	N	N	Y	N	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
67	SS	Y	Y	N	N	N	Y	Y	Y	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
68	HK	Y	N	N	N	Y	Y	Y	N	Cavitatory lesions		E	IA-Probable	Possible
69	SR	N	N	N	N	N	N	N	N	Cavitatory lesions		E	IA-Probable	Possible
70	SB	Y	N	N	N	N	N	N	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
71	GK	N	N	N	N	N	Y	Y	N	Homogenous haziness		E	IA-Probable	Possible
72	GS	Y	N	N	N	N	Y	N	Y	Cavitatory lesions		E	IA-Probable	Possible
73	GS	Y	N	N	N	N	Y	N	Y	Cavitatory lesions	Consolidation	D	IA-Probable	Possible
74	HS	Y	N	N	N	Y	N	N	N	Cavitatory lesions		D	IA-Probable	Possible
75	IS	Y	Y	N	N	N	N	Y	N	Cavitatory lesions		D	IA-Probable	Possible
76	CK	Y	Y	N	N	N	Y	Y	N	Homogenous haziness		D	IA-Probable	Possible
77	RK	Y	Y	N	N	N	N	N	N	Homogenous haziness		D	IA-Probable	Possible
78	SS	N	N	N	N	N	N	N	N	Homogenous haziness	Consolidation	E	ABPA	
79	HS	Y	Y	Y	N	N	Y	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
80	AS	Y	Y	N	N	N	N	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
81	LJ	Y	N	N	Y	N	Y	Y	Y	Homogenous haziness		D	IA-Probable	Possible
82	SK	Y	N	N	N	N	N	Y	N	NAD		D	Colonizers	
83	KS	N	Y	N	Y	N	N	N	N	Homogenous haziness		D	IA-Probable	
84	BS	N	Y	N	N	N	Y	Y	Y	Cavitatory lesions	Homogenous haziness	D	IA-Probable	Possible
85	LJ	Y	Y	N	N	N	Y	Y	N	NAD		D	Colonizers	
86	RK	N	N	N	N	N	Y	Y	Y	Homogenous haziness	Consolidation	D	IA-Probable	Possible
87	MD	Y	N	N	N	N	Y	N	Y	NAD		D	IA-Probable	Possible
88	PL	Y	N	N	N	N	Y	Y	Y	Homogenous haziness	Consolidation	E	IA-Probable	Possible
89	NS	Y	N	N	N	N	Y	Y	Y	Cavitatory lesions	Consolidation	E	IA-Probable	Possible

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The Principal,
DMC,Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 22.01.2021 has approved the plan of thesis of **Dr. Bavleen Kaur**, a student of your college for **MD Microbiology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Clinico-mycological profile of suspected pulmonary aspergillosis cases

Supervisor
Dr. Veenu Gupta
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DMCH, Ludhiana.

Co-Supervisor
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Dept. of Microbiology,

The student has to submit the three copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

1. ***If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.***
2. *The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination*

CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

In

MICROBIOLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

November, 2019

Dr Bavleen Kaur

DEPARTMENT OF MICROBIOLOGY
DAYANAND MEDICAL COLLEGE AND HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES
For the degree of	MD (Microbiology)
Name of the candidate	Dr. Bavleen Kaur
Supervisor	Dr. Veenu Gupta, Professor Department of Microbiology, DMCH, Ludhiana
Co-supervisor	Dr. Anil Kumar Kashyap, Associate Professor, Department of Pulmonary Medicine, DMCH, Ludhiana. Dr. Jyoti Chaudhary, Associate Professor, Department of Microbiology, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Aspergillosis, can be defined as an infection or disease caused by fungi in the genus *Aspergillus*. It constitutes a wide variety of disease entities that form a continuum ranging from allergic reactions to disseminated invasive disease.With increase in the number of immunocompromised patients, there has been a dramatic increase in severe and fatal invasive aspergillosis.

Aim of study: 1. To study the clinical spectrum and characterization of pulmonary Aspergillosis. 2. To correlate mycological and serological profile of suspected pulmonary Aspergillosis cases.

Materials and Methods: The present study will be a prospective study conducted over a period of one year in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana.

Various samples from patients suspected of pulmonary aspergillosis will be collected. Various tests like fungal smear and culture /galactomannan assay/ (1-3)-ß-D Glucan Assay/ serum IgE levels/ aspergillus skin test will be done. These cases will be characterized on the basis of clinical aspects and outcome.

Clinical significance: Incidence of fungal infections have been increasing. Timely diagnosis relies on appropriate use of laboratory testing, including antigen testing and serological testing. This can lead to decrease in both morbidity and mortality.

Keywords: Fungal smear, Fungal culture, ABPA(Allergic Bronchopulmonary Aspergillosis), Invasive aspergillosis, Aspergilloma.

Bavleen kaur — (Signature) Candidate

Jyoti Chaudhary — (Signature) Co-supervisor

Anil — (Signature) Co-supervisor

Veenu — (Signature) Supervisor

(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES"** exist in the Department of MICROBIOLOGY, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

Supervisor

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Co-Supervisor(s)

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Place : _____

Date : _____

APPROVAL PROFORMA
BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Bavleen Kaur
Department	Microbiology
Topic of Thesis	CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES
Likely date of appearing in PG examination	May 2022
Date of enrollment	
Name of Head of Department	Dr. Deepinder K Chhina
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Jaspal Singh (Professor & Head, Surgery)	Mr. A K Jindal (Member, Ethics Committee)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Assistant Dean Academics)	Dr. Deepinder K Chhina (Professor & Head, Microbiology)
Dr. Rajoo Singh Chhina (Dean Academics)	Dr. Neena Sood (Professor & Head, Pathology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / ~~No~~

Dr. Sandeep Puri
Chairperson
Research Committee

Chairperson
Research Committee

Approved: Yes / ~~No~~

Prof. Arvind Malhotra
Chairperson
Ethics Committee

Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	DR BAVLEEN KAUR 230-MODEL GRAM LUDHIANA,PUNJAB
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL
3	COURSE OF STUDY AND SUBJECT	MD MICROBIOLOGY
4	DATE OF ADMISSION OF COURSE	18-05-2019
5	TITLE OF THE TOPIC	CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES.

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR STUDY

Fungal infections have recently emerged as a world-wide healthcare problem, owing to extensive use of broad spectrum antibiotics, immunosuppressive agents and increasing population of terminally ill and debilitated patients.[1] Aspergillus infection is the commonest invasive fungal infection which involves respiratory tract. Delayed diagnosis and therapy may lead to poor outcomes. Therefore, early diagnosis and appropriate antifungal therapy is important in reducing mortality and morbidity due to aspergillosis.

The rising prevalence of fungal infections is likely related to several host factors, including immunocompromised individuals with malignancies, solid organ transplants, HIV and inflammatory conditions treated with immunosupressants.[2] In addition to heightened awareness, recent advances in laboratory diagnostics have evolved to assist the diagnosis of pulmonary fungal infections. These methods include antigen testing in blood, fungal smear or fungal culture of BAL, ET and sputum, serological testing to detect antibodies to fungal components.[2]

The purpose of this study is to review available information for the diagnosis of aspergillosis infections using the laboratory approaches. The clinician must also consider the immune status of the patient when weighing the possibility of fungal infection. Although most clinicians consider fungal lung infections in the severely immune-compromised host, such as those with profound neutropenia (500/µl), hematological or solid malignancy, or poorly controlled HIV (with CD4 counts 200/µl), additional emerging immune-suppressed groups are also at risk for fungal infections.[3]

Moreover, prolonged use of broad spectrum antibiotics in chronic lung disease patients may stimulate the growth and virulence of infecting fungi by destruction of competing bacterial flora.[4]

Clinical diagnosis of aspergillosis is often difficult to make, as clinical signs and symptoms are not specific and as they are consistent with bronchopneumonia – fever unresponsive to antibiotics, cough, sputum production and dyspnea.[5] Radiological findings are also of little diagnostic help, especially in early stages of disease, because of the presence of non-specific changes. Usual findings include rounded opacities, pleural-bases infiltrates and cavitations. Typical 'halo sign' and 'crescent sign' which can be seen in invasive disease are less specific and they can be found in other fungal and bacterial infections.[6] Histopathological examination is an invasive procedure, though it is confirmatory, it cannot be done in all cases.

Aspergillus can be identified in the direct specimens microscopically on detecting the presence of hyaline, regular, slender, septate hyphae with dichotomous branching (45°).[5] *Aspergillus* can cause a variety of clinical manifestations depending on the immune status of the host. For example, allergic bronchopulmonary aspergillosis (ABPA), aspergilloma which is mainly observed in patients with chronic cavitary lung disease. Other forms of aspergillosis include chronic necrotizing pulmonary aspergillosis, which is seen in immunocompromised patients and those individuals with chronic lung diseases, and invasive pulmonary aspergillosis (IPA), which is the most serious lung infection due to *Aspergillus* spp. and typically occurs only in severely immunocompromised patients.

A high index of suspicion must be maintained to detect invasive fungal infection in the patients with severe and moderate immune suppression and in those with exposure to these pathogens. Therefore the need of my study lies on the fact that study of diagnostic tests and their correlation with the clinical condition of the patient will impact the patient outcome with regard to morbidity and mortality.

6.2 REVIEW OF LITERATURE

Aspergillosis, which can be defined as an infection or disease caused by fungi in the genus *Aspergillus* constitutes a wide range of disease entities that form a continuum from allergic reactions to disseminated invasive disease.[7] The specific term invasive aspergillosis (IA), often defined in relation to the primary affected organ as invasive pulmonary aspergillosis (IPA)is commonly considered to be defined by invasion of the pulmonary parenchyma by the growing hyphae of Aspergillus.[8]

Fungal infections have recently emerged as a world-wide health care problem, owing to extensive use of broad spectrum antibiotics, immunosuppressive agents and increasing population of terminally ill and debilitated patients. *Aspergillus* infection is the commonest invasive fungal infection which involves respiratory tract. It can be ascribed to pervasive presence of the fungal spores in soil, water, decaying vegetation, indoor environment, including hospitals. *Aspergillus* was first detected in 1729 and it received its name due to its resemblance to an aspergillum which was used to sprinkle holy water.[5] Man acquires the infection through inhalation of fungal spores, though infection has also been documented following exposure to water aerosols contaminated with *Aspergillus* spores and *A. fumigatus*, in particular, is the most important air borne saprophytic fungus[9]. Inhalation being the commonest mode of infection, lung is the most common site of involvement and pneumonia is the most important nosocomial infection caused by *Aspergillus*.[4] Depending on the host immunity, lung structure and degree of inoculums, the spectrum of pulmonary disease ranges from a noninvasive disease (colonization or aspergilloma), or an allergic bronchopulmonary aspergillosis (ABPA), to a semi-invasive (chronic necrotizing pneumonia) or an invasive infection (invasive pulmonary aspergillosis).[10] Dissemination of infection from lungs can lead to systemic aspergillosis, that can be a major cause of morbidity and mortality in immunosuppressed persons.

Pulmonary aspergillosis is seen mostly in immunosuppressed individuals. The central cause is the impairment of phagocytic system and hence, patients with haematological disorders are particularly prone to develop it. Diabetes mellitus, alcohol abuse, renal and hepatic failure, etc. which have an effect on neutrophil proliferation, maturation, function and lifespan, are the important predisposing factors.[5] The incidence of *Aspergillus* infection is showing a rising trend, following solid organ or bone marrow transplantation, chemotherapeutic modalities like prolonged use of corticosteroids or other immunomodulating drugs and epidemic occurrence of human immunodeficiency virus (HIV).[11] *A. fumigatus* is the commonest species causing infection, though other species like A. flavus, *A. niger* and *A. terreus* can also cause the disease less frequently.[12]

Pre-existing lung diseases like bronchial asthma, bronchiectasis, COPD (Chronic obstructive pulmonary diseases) unresolved pneumonia, lung abscess, tuberculosis, malignancy, pulmonary edema due to CCF, act as predisposing factors along with other conditions like diabetes mellitus, great use of broad spectrum antibiotics, corticosteroids, immune suppressants or cytotoxic drugs.[13] In the lungs, the lesions caused by *Aspergillus* species are allergic broncho pulmonary aspergillosis, aspergilloma in a cavity lesion, chronic necrotizing aspergillosis, invasive aspergillosis which is associated with high morbidity and mortality.Other lesions caused by *Aspergillus* species are keratitis, otomycosis, onychomycoses, paranasal sinus granuloma, endocarditis.[13] The incidence of *Aspergillus* infections is on the rise due to increased longevity, increase in lung diseases due to pollution, improved survival from other diseases like AIDS, organ transplants etc.

Invasive aspergillosis cases will be classified according to revised definitions given by EORTC/MSG Consensus group into proven, probable and possible invasive aspergillosis based on host, clinical and mycological factors. Invasive aspergillosis (IA), an infection, caused by fungi in the genus *Aspergillus*, is seen in patients with immunological deficits,

particularly acute leukaemia and stem cell transplantation, and has been associated with high rates of mortality in previous years.[10] Diagnosing IA has long been problematic owing to the inability to culture the main causal agent *A. fumigatus* from blood. Microscopic examination and culture of respiratory tract specimens have lacked sensitivity, and biopsy tissue for histopathological examination is rarely obtainable. Thus, for many years there has been a great interest in nonculture-based techniques such as the detection of galactomannan, β-D-glucan, and DNA by PCR-based methods.[14] A multicenter prospective study comparing 170 patients without signs of invasive fungal infection and 163 patients with proven (142 patients) or probable (21 patients) invasive fungal infection found a 64.4% sensitivity, 92.4% specificity, 89% positive predictive value, and 73% negative predictive value of BG assay.[15] The specific term invasive aspergillosis (IA), often defined in relation to the primary affected organ as invasive pulmonary aspergillosis (IPA) is commonly considered to be defined by invasion of the pulmonary parenchyma by the growing hyphae of *Aspergillus*. The most common aetiological agent of IA, *A. fumigatus*, is a ubiquitous fungus, with airborne conidia leading to almost universal and constant exposure in almost all humans.

Identification of *A. fumigatus* is based predominantly upon the morphology of the conidia and conidiophores. The organism is characterized by green echinulate conidia, 2.5 to 3 µm in diameter, produced in chains basipetally from greenish phialides, 6 to 8 by 2 to 3 µm in size. A few isolates of *A. fumigatus* are pigmentless and produce white conidia.[16]

ABPA is currently the most severe allergic pulmonary complication caused by *Aspergillus* species. It occurs in patients suffering from atopic asthma or cystic fibrosis. ABPA occurs in approximately 1 to 2% of asthmatic patients.[17] It follows the same course as classic asthma, with a unique cellular immune response and pathophysiologic findings caused by the response of T-cell products.[18] Its effects range from asthma to fatal destruction of the lungs with defined clinical, serological, radiological, and

pathological features.[19] Clinically, ABPA manifests as a bronchial asthma with transient pulmonary infiltrates that may proceed to proximal bronchiectasis and lung fibrosis.[20] It is a very difficult syndrome to diagnose. The criteria classically listed for a definitive diagnosis is asthma, peripheral blood eosinophilia (1,000/µl), immediate skin reactivity to *A. fumigatus* and IgE antibodies against *A. fumigatus*, elevated levels of total IgE in serum, a history of pulmonary infiltrates, and central bronchiectasis. Aspergilloma, commonly referred to as "fungus ball," occurs in preexisting pulmonary cavities that were caused by tuberculosis, sarcoidosis, or other bullous lung disorders and in chronically obstructed paranasal sinuses.[21] A common symptom of aspergilloma is hemoptysis. Hemoptysis results from the disruption of blood vessels in the wall of the cavity occupied by the fungus or in the bronchial artery supply, centimeters away from the aspergilloma.[22] Patients are usually asymptomatic, and aspergillomas are most often detected on chest radiographs obtained for the evaluation of another pulmonary or allergic disease.

There are now expanded options of laboratory approaches to confirm the suspected diagnosis of fungal lung infection. Direct microscopic examination of endotracheal secretions, BAL, and tissue sampling often provides the most rapid means to confirm the diagnosis. Such examination can be performed after traditional KOH smear. The advent of a variety of antibody reagents has also enhanced the usefulness of direct and indirect immunofluorescence in the diagnosis of various fungal infections. Serological testing detects host antibody reactivity to various fungal components. More recently, specific antigen components of the fungal cell wall have been exploited for the development of diagnostic assays that can detect the presence of these components in the serum (e.g., galactomannan assays in the diagnosis of invasive Aspergillus infection) or BAL (e.g., newer uses of galactomannan assays for *Aspergillus*.[2] Early and accurate diagnosis of IA is essential in order to start timely antifungal therapy and has an impact on the prognosis.

6.3 AIMS & OBJECTIVES OF THE STUDY

- To study the clinical spectrum and characterization of pulmonary Aspergillosis.
- To correlate mycological and serological profile of suspected pulmonary Aspergillosis cases.

7. MATERIALS AND METHODS

The present study will be a prospective study conducted over a period of one year in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana.

Samples like sputum, Bronchoalveolar lavage (BAL), endotracheal secretions, bronchial brushings, specimens obtained by needle aspiration or biopsy and blood (for serological analysis) from patients suspected of pulmonary aspergillosis will be included in our study.

To rule out the chances of contamination, samples with repeated isolates of *Aspergillus* (minimum two sputum samples or one sputum and one BAL) will be included in the study.

Sample Processing:

MYCOLOGICAL EVALUATION

1. **Direct examination (Fungal smear):** A drop of 10-20% KOH will be put on the center of the clean glass slide. Then the specimen will be placed in the KOH drop and mixed gently by inoculating loop. A cover slip will be placed over the specimen. The slide will be observed under high power objective.

2. **Fungal culture:** Specimen will be inoculated on 2 sets of Sabouraud's dextrose agar(SDA) with and without cycloheximide and incubated at 25°C and 37°C . Growth obtained on SDA will be identified by LCB preparation.

SEROLOGICAL EVALUATION

1. **Aspergillus skin test (Intradermal test):** It is performed by using Aspergillus antigen kit as per the kit manual.

2. **Total IgE levels:** The test will be performed on serum or plasma using enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) technique by COBAS e411-ECL as per protocol.

Interpretation:

Age group	IU/ml
Neonates	<1.5

Infants	<15
Children (1-5 years)	<60
Children (6-9 years)	<90
Children (10-15 years)	<200
Adults	>100

3. **Galactomannan assay**: Immunoenzymatic sandwich microplate assay which detects galactomannan in human serum. Uses rat EBA-2 monoclonal antibodies.

Interpretation:

NEGATIVE RESULT: <0.50

POSITIVE RESULT: ≥0.50

4. **(1-3)-β-D Glucan Assay**: The Fungitell kinetic assay is based upon the determination of the rate of optical density increase produced by a sample. The Fungitell test results are expressed in picogram/ml.

Interpretation:

NEGATIVE RESULT: <60 pg/mL

INDETERMINATE RESULT: to 79 pg/mL

POSITIVE RESULT: ≥ 80 pg/mL

RADIOLOGICAL EVALUATION:

Chest X-Ray and High Resolution Computerized Tomography (HRCT)

7.1 SOURCE OF DATA

All samples received from patients with suspicion of pulmonary aspergillosis in Dayanand Medical College and Hospital will be included.

7.2 METHOD OF COLLECTION OF DATA

Inclusion Criteria:

All patients of suspected pulmonary aspergillosis become positive by any of these methods, fungal smear/ fungal culture/ galactomannan antigen/ (1-3)-β-D- Glucan /aspergillus skin test/ total IgE levels.

Data collected will be entered in a pre structuredproforma which will include patient's details: Name, age, gender; MRD number, Lab ID No., date of admission, presenting complaints, sample, predisposing risk

factors, treatment history, microbiological evaluation { fungal smear/ culture, (1-3)-β-D- Glucan, galactomannan, aspergillus skin test, total IgE levels },absolute eosinophil count (AEC), chest X-ray and high resolution computerised tomography (HRCT) (if required), treatment history and outcome.

Outcome:

Pulmonary aspergillosis will be classified as Invasive aspergillosis, ABPA and Aspergilloma. Clinical spectrum and mycological findings of suspected pulmonary Aspergillosis cases will be correlated in our study.

Statistical analysis:

Data will be described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

Not Applicable

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached Herewith

8. LIST OF REFERENCES

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PROFORMA

Sr. No.

Name: Lab No:

Age/Sex: Date of admission:

MRD No: Ward:

Admission No:

Date of sample collection:

Specimen:Sputum/BAL/ET/Serum

Provisional Diagnosis:

Chief complaints:

History of present illness:

Candidate Signature **Supervisor Signature**

Examination

Risk factors: Suspected pulmonary infection/Malignancy/Solid organtransplant/HIV/Immunocompromised/Steroid therapy/Neutropenia Others.

Treatment history:

Investigations: Hb/TLC/DLC/Eosinophil count

Radiological evaluation:

Microbiological evaluation:

- Fungal smear/Culture:
- Galactomannan assay:
- (1-3)-β-D glucan assay:
- IgE levels:

Outcome:

Others:

Candidate Signature **Supervisor Signature**

INFORMED PATIENT CONSENT

Date: _____

I.....D/o, W/o, S/o..... .....am willing to participate in the study **"CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

.....
Signature of the Witness

Name:

.....
Signature of the patient

Signature of Candidate

Signature of Supervisor

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

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गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

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अन्वेषक के हस्ताक्षर

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विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

"CLINICO-MYCOLOGICAL PROFILE OF SUSPECTED PULMONARY ASPERGILLOSIS CASES" ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

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ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ

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ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____

ਪਤਾ _____

ਮਿਤੀ_____

ਪਤਾ _____

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ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

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ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ